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Hexokinase Isoenzymes from Anaplastic and Differentiated Medullary Thyroid Carcinoma in the Rat*

GERT RIJKSEN, RALPH OSKAM, CARLA F. M. MOLTHOFF, SUE-JIN LEE ON, MARIANNE STREEFKERK and GERARD E. J. STAAL

Division of Medical Enzymology, Department of Haematology, Academic Hospital, Catharijnesingel 101, 3500 CG Utrecht, The Netherlands

Abstract—*The activity, isoenzyme distribution and compartmentation of hexokinase (ADP: D-hexose-6-phosphotransferase, EC 2.7.1.1) were compared in slowly growing, well-differentiated medullary thyroid carcinoma (DMTC) and rapidly proliferating anaplastic thyroid carcinoma (AMTC) in the rat. Individual isoenzymes from either soluble or particulate fractions after solubilization were obtained by fast protein liquid chromatography and were kinetically analyzed either in soluble form or after (re)binding to rat liver mitochondria. These studies were undertaken to test the hypothesis that the growth rate of tumors is correlated with the activity of mitochondrial-bound hexokinase in our tumor system. In contradiction to this hypothesis, we found no difference in either enzyme activity or compartmentation of both kinds of tumors. The major part of enzyme activity was soluble (73 and 78% in DMTC and AMTC respectively). In addition, no major differences were observed in the kinetic properties of the individual isoenzymes of both tumors. Only soluble type II hexokinase from AMTC had a slightly decreased apparent K_m for glucose. There appeared to be some differences in isoenzyme composition: both tumors contained type I and type II hexokinase in the soluble as well as in the particulate fractions. However, the proportion was shifted in favor of type II hexokinase in the soluble fraction of AMTC. Additional findings of this study were the following: the affinity of type II hexokinase to both substrates glucose and $MgATP^{2-}$ was significantly less compared to type I hexokinase. However, the inhibition constant for glucose-1,6-diphosphate of both isoenzymes was exactly the same. The bound form of both isoenzymes had the same substrate affinities as the soluble form but was considerably less inhibited by glucose-1,6-diphosphate. In the latter respect, type I and type II hexokinase behaved in the same way.*

INTRODUCTION

HEXOKINASE (ADP: D-hexose-6-phosphotransferase, EC 2.7.1.1) is one of the key enzymes of glycolysis. In some tissues it can exert its regulatory function through a rather unique 'ambiquitous' behavior [1], i.e. its activity is determined by the intracellular localization, being either cytosolic or particulate.

Particulate hexokinase has been shown to be bound to a specific binding protein at the outer surface of the mitochondrial membrane [2]. The bound enzyme is less inhibited by phosphorylated hexoses than the soluble form and can directly benefit from the ATP produced by the mitochondrial respiration for the phosphorylation of glucose. For these reasons it was suggested that a high activity of bound hexokinase is in favor of an active glycolytic pathway [1].

In general, neoplastic tissues have been characterized by a high glycolytic capacity. The high glycolytic rate in hepatoma cells was shown to be due, at least in part, to an elevated activity of hexokinase in the mitochondrial fraction [3, 4]. In addition, the growth rate of a series of Morris- and other hepatomas was positively correlated with the mitochondrial hexokinase activity [4].

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Although in many reports information about the isoenzyme composition is lacking, most investigations comparing the normal and neoplastic tissues concern the type I isoenzyme [1, 5], the most predominant isoenzyme of hexokinase in mammalian tissues. However, isoenzyme shifts of hexokinase in neoplastic tissues have been reported, e.g. in uterus carcinomas [6] and gliomas [7] hexokinase type II was detected, an isoenzyme absent in the normal tissues from which these tumors originate. Hepatomas also contain considerable amounts of hexokinase type II, in addition to the isoenzymes I and III [8, 9]. The type II isoenzyme can be present in particulate form, too [10, 11], but fewer data are available concerning its binding ability and the kinetic properties of its soluble and bound forms. It seems obvious that differences in kinetic properties found between bound and soluble hexokinase might easily be misinterpretated when more than one isoenzyme is present.

In order to study whether differences in growth rate and glycolytic capacity between tumors might be reflected in changes in composition and kinetics of hexokinase isoenzymes, we compared the compartmentation and isoenzyme distribution of hexokinase from well-differentiated (DMTC) and anaplastic medullary thyroid carcinoma (AMTC) in the rat and studied the kinetic properties of the separate bound and soluble isoenzymes.

MATERIALS AND METHODS

MTC tumors in Wag/Ry rats

Medullary thyroid carcinomas (MTC) were passaged in rats of the Wag/Ry strain by implantation of small sections of the tumor (1 mm^3) under the renal capsule. The tumors reached an average size of approx. 5 g within 3 and 20 weeks, respectively, for anaplastic (AMTC) and differentiated (DMTC) tumors. At that time the tumors were surgically removed under ether anesthesia and blood samples were taken from the orbital plexus and collected in EDTA (2 mM final concentration). Tumors were either immediately frozen in liquid nitrogen and stored at -70°C or processed freshly. AMTC were microscopically proven to be anaplastic according to Singer and Habener [12]. DMTC produced large amounts of peptide hormones, e.g. calcitonin and β-endorphin, while AMTC secreted only trace amounts of peptide hormones [13].

Materials

DEAE-Sephadex A-50 and a Mono Q™ HR 5/5 column were obtained from Pharmacia (Uppsala, Sweden). The latter column was used in connection with the Pharmacia fast protein liquid chromatography system.

Substrates, inhibitors, auxiliary enzymes for the hexokinase assay, and the lactate testkit were purchased from Boehringer (Mannheim, F.R.G.). All other chemicals were analytical grade and were obtained either from Merck (Darmstadt, F.R.G.) or from BDH Chemical Ltd. (Poole, U.K.).

Preparation of extracts

Tumors were dissected free of necrotic parts, sometimes present in AMTC, and haemorrhages, usually present in DMTC. The tissue was homogenized in a Waring Blendor and a Potter homogenizer, successively, in 4 vols of an isotonic extraction buffer containing 10 mM Tris–HCl (pH 7.4 at 20°C), 1 mM EDTA, 1 mM dithiothreitol, 10 mM ε-aminocaproic acid and 0.25 M sucrose (buffer A), to which 1 mM diisopropylfluorophosphate was added immediately before use. Cell debris was removed by centrifugation for 10 min at 800 ***g***.

Compartmentation studies

Bound hexokinase was prepared by centrifuging the homogenate for 15 min at 48,000 ***g***. The resulting supernatant is referred to as the cytosolic fraction containing soluble hexokinase. The pellet fraction, referred to as the 'particulate fraction', contained bound hexokinase and was resuspended in buffer A supplemented with 10 mM $MgCl_2$ to prevent spontaneous solubilization. Before determination of soluble and bound hexokinase activity both fractions were incubated for 30 min with 0.5% Triton X-100, which did not influence enzyme activity.

Solubilization of bound hexokinase

Hexokinase was solubilized by incubation of the particulate fraction in buffer A supplemented with either 1 mM glucose-6-phosphate or 0.5 M KCl (in the absence of $MgCl_2$) for 15 min at 25°C. Subsequently, the mixture was centrifuged for 15 min at 48,000 ***g***. The pellet fraction was washed twice and the supernatant fractions were combined. In both cases (glucose-6-phosphate as well as KCl) 75–90% of bound activity could be solubilized.

Separation of isoenzymes

Soluble hexokinase was partially purified by batchwise treatment with DEAE-A-50 and concentrated by $(NH_4)_2SO_4$ precipitation as previously described [14]. The precipitate was dissolved in 10 mM Tris–HCl buffer, pH 7.0, containing 10 mM KCl, 10 mM glucose and 3 mM β-mercaptoethanol, dialyzed against the same buffer and subsequently subjected to fast protein

liquid chromatography (FPLC) on a Mono Q™ HR 5/5 column. Mono Q is a strong anion exchanger which enables very fast protein separations with high resolution. Enzyme fractions were eluted by applying an NaCl gradient, as shown in Fig. 1. Hexokinase isoenzyme types I and II were completely separated within 15 min. Isoenzymes from solubilized fractions were separated by FPLC without pretreatment with DEAE-A-50. The isoenzymic nature of the activity peaks was determined electrophoretically.

(Re)binding experiments

Rat liver mitochondria do not contain bound hexokinase activity but do possess the ability to bind hexokinase [2]. Therefore experiments involving the binding of soluble hexokinases or rebinding of solubilized hexokinases were performed with the mitochondrial fraction from rat liver, prepared as decribed above for tumor tissues. The liver mitochondrial fraction was added to excess of either soluble or solubilized hexokinase isoenzymes and dialyzed overnight against buffer A supplemented with 10 mM $MgCl_2$. After dialysis the samples were centrifuged (48,000 *g*, 10 min, 4°C) and the pellet fractions were resuspended in buffer A, supplemented with 10 mM $MgCl_2$ to prevent spontaneous solubilization. Total bound activity was determined after incubation for 30 min with 0.5% Triton X-100.

Hexokinase assay and kinetic studies

Hexokinase activity was measured at 37°C in a glucose-6-phosphate dehydrogenase coupled assay, as described previously [14]. Activities are expressed in units/mg protein, whereas one unit is defined as the amount of enzyme which catalyzes the formation of 1 μmol of glucose-6-P per min at 37°C. Protein content was determined by the method of Lowry *et al.* [15], using bovine serum albumin as a standard. Apparent K_ms of hexokinase isoenzymes for the substrates glucose and $MgATP^{2-}$ were determined at the substrate concentrations indicated in the text. Excess of free Mg^{2+} over $MgATP^{2-}$ was kept constant at 5.0 mM in all conditions. Inhibition studies with glucose-1,6-diphosphate were performed at 7 mM glucose and 0.35 mM $MgATP^{2-}$ (pH 7.15, 37°C). In some cases true inhibition constants for glucose-1,6-diphosphate were determined by varying $MgATP^{2-}$ in the range of 0.2–5.0 mM and glucose-1,6-diphosphate in the range of 0–0.4 mM. The results were fitted into either a non-competitive or a competitive model by using the COMP and NONCOMP computer programs described by Cleland [16]. The kinetic constants of the different isoenzymes in the different fractions were compared by using Student's *t* test. Bound hexokinase was checked for spontaneous solubilization by centrifugation immediately before the assays. Any spontaneously solubilized hexokinase was removed. No solubilization appeared to occur during the assay.

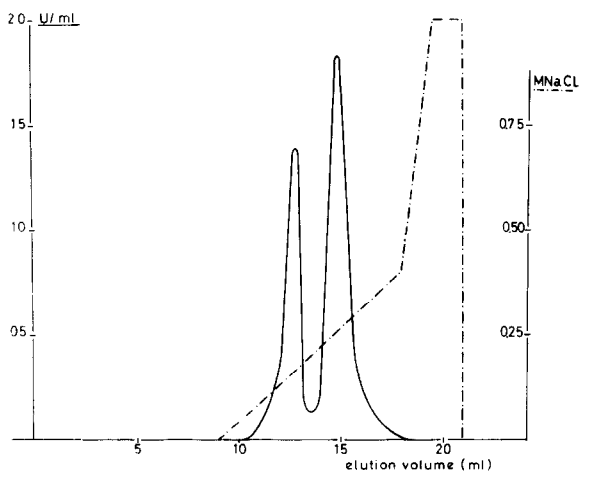

Fig. 1. Separation of hexokinase type I and II from AMTC by FPLC on a Mono Q™ column. The column was loaded with 20–30 mg *of protein and eluted at a flow rate of* 1.5 ml/min.

Hexokinase electrophoresis

Electrophoresis on cellulose acetate was performed as described before [17] except that the agar was omitted from the staining mixture.

Determination of lactic acid

The contents of lactic acid of plasma and of freshly prepared tumor homogenates (48,000 *g* supernatants) were measured in an enzyme-coupled assay by using a testkit.

RESULTS

Glycolytic activity

The *in vivo* glycolytic activity of the tumors was estimated by the determination of the lactic acid levels in tumors and in plasma of tumor-bearing animals (Table 1). The plasma levels were increased in rats bearing DMTC compared to non-cancerous animals ($P < 0.05$ with Student's *t* test) and even more in AMTC-bearing rats

Table 1. Contents of lactic acid in AMTC and DMTC and in plasma from tumor-bearing animals and controls

	Plasma levels mmol/l	Tumor contents ng/mg soluble protein
Controls	1.37 ± 0.23 (7)	—
DMTC	1.91 ± 0.64 (12)	11.1 ± 3.0 (7)
AMTC	3.75 ± 0.93 (6)	15.5 ± 2.4 (8)

Values are means ± S.D. with the number of samples in parentheses.

($P < 0.01$ with respect to DMTC). It is conceivable that these data can hardly be used as evidence for an increased *in vivo* glycolytic activity in AMTC vs DMTC, because differences in tumor mass may exist. However, the lactic acid content of AMTC expressed per mg of soluble protein was also significantly increased ($P < 0.01$) with respect to DMTC, which is highly indicative for an increased *in vivo* glycolytic activity in AMTC.

Activity and intracellular localization of hexokinase

The specific activities of hexokinase in DMTC and in the greater part of the AMTC are the same. However, the activities in the group of AMTC are not normally distributed. One particular subgroup with about four times higher activities can be discerned (Table 2). This subgroup, which represents approx. 15% of all AMTC, cannot be distinguished by any other biochemical or histological parameter thus far investigated and shows no aberrant growth characteristics.

Table 2. Hexokinase activity and intracellular localization

	Activity U/mg protein	% soluble
DMTC	0.097 ± 0.051 (26)	73 ± 9 (26)
AMTC I	0.080 ± 0.039 (24)	78 ± 8 (24)
II	0.31 ± 0.057 (5)	77 ± 11 (5)

The values are means ± S.D. with the number of tumors in parentheses.

In all tumors the greater part of hexokinase activity is found in the soluble fraction and no difference is observed between DMTC and AMTC.

Hexokinase isoenzyme distribution

The isoenzyme distribution of soluble and solubilized hexokinase from AMTC and DMTC was studied by cellulose acetate electrophoresis. A rat liver extract was used as a reference. Three bands of hexokinase activity are present in liver, representing the three 'low K_m' hexokinases I, II and III, numbered in order of increasing anodal mobility. The results of the densitometric analysis of the electropherograms are summarized in Table 3. All three isoenzymes are present in the soluble fraction of both AMTC and DMTC, although the amount of type III hexokinase is only minor. The main isoenzyme in both tumors is type I hexokinase; however, a slight but significant ($P < 0.01$) shift in favor of type II hexokinase is observed in the cytosolic fraction of AMTC with respect to that of DMTC. The particulate fraction—i.e. solubilized hexokinase—of both tumors is slightly enriched in type I hexokinase with respect to the soluble fraction and no bound type III hexokinase is found. The isoenzyme distribution of the particulate fraction of AMTC and DMTC is the same (Table 3).

Kinetic properties

The kinetic parameters of soluble and bound isoenzymes from DMTC and AMTC are summarized in Table 4 (A and B).

The kinetic properties of hexokinase type I as well as type II are the same in AMTC and in DMTC, with the possible exception of the K_m glucose of soluble type II hexokinase, which is slightly lower ($P < 0.05$) in AMTC than in DMTC.

When the kinetic constants of types I and II hexokinase are compared mutually, one can observe not only a difference in K_m glucose ($P < 0.01$ for the soluble enzymes and $P < 0.02$ for the bound forms), but also a highly significant difference in the affinity for the substrate $MgATP^{2-}$ ($P < 0.001$ for the soluble enzymes and $P < 0.02$ for the bound forms). Type I hexokinase is apparently less inhibited by glucose-1,6-diphosphate than type II hexokinase, at least at one fixed $MgATP^{2-}$ concentration. However, this difference is brought about by the difference in K_m $MgATP^{2-}$. When true inhibition constants were determined, exactly the same values were obtained for both type I and type II hexokinase ($K_i = 0.030$ mM, S.E. = 0.003 and $K_i = 0.029$ mM, S.E. = 0.002

Table 3. Isoenzyme distribution

Hexokinase		Type I (%)	Type II (%)	Type III (%)
DMTC — soluble	(13)	65 ± 13	29 ± 10	6 ± 4
— solubilized	(9)	81 ± 7	19 ± 7	—
AMTC — soluble	(10)	53 ± 9	43 ± 11	5 ± 4
— solubilized	(9)	77 ± 11	23 ± 11	—

Values are means ± S.D. with the number of determinations in parentheses, and are obtained by densitometric analysis of the electrophoretic patterns.

Table 4a. Kinetic parameters of soluble and bound hexokinases from DMTC

Isoenzyme	K_m app glucose (mM)	K_m app $MgATP^{2-}$ (mM)	K_i app Glc-1,6-P_2 (mM)
Type I soluble	0.072 ± 0.015 (5)	0.50 ± 0.10 (12)	0.084 ± 0.017 (9)
Type I bound	0.061 ± 0.013 (5)	0.50 ± 0.17 (9)	0.22 ± 0.11 (5)
Type II soluble	0.19 ± 0.08 (4)	0.82 ± 0.22 (9)	0.032 ± 0.018 (5)
Type II bound	0.23 ± 0.10 (4)	0.62 ± 0.11 (3)	0.14 ± 0.04 (4)

Table 4b. Kinetic parameters of soluble and bound hexokinases from AMTC

Isoenzyme	K_m app glucose (mM)	K_m app $MgATP^{2-}$ (mM)	K_i app Glc-1,6-P_2 (mM)
Type I soluble	0.057 ± 0.017 (9)	0.45 ± 0.10 (12)	0.070 ± 0.020 (11)
Type I bound	0.071 ± 0.021 (5)	0.44 ± 0.20 (8)	0.18 ± 0.05 (10)
Type II soluble	0.11 ± 0.04 (10)	0.87 ± 0.15 (12)	0.040 ± 0.018 (10)
Type II bound	0.16 ± 0.10 (4)	0.76 ± 0.26 (10)	0.15 ± 0.02 (5)

Individual isoenzymes were obtained by subjecting soluble or solubilized activity from DMTC and AMTC to fast protein liquid chromatography. Peak fractions were investigated either in soluble form or after binding to rat liver mitochondria as described in Materials and Methods. The apparent K_m glucose was determined at a concentration of 5 mM $MgATP^{2-}$, the K_m app $MgATP^{2-}$ at 10 mM glucose. The apparent K_i for glucose-1,6-diphosphate ($K_i$app Glc-1,6-P_2) represents the concentration required for 50% inhibition at a concentration of 0.35 mM $MgATP^{2-}$ and 7 mM glucose (pH 7.15, 37°C). The values represent the mean ± S.D., whereas the number of independent determinations are indicated in parentheses.

respectively for the soluble isoenzymes from AMTC).

No statistically significant differences in substrate affinity could be discerned when the soluble and bound forms of both isoenzymes were compared. However, the bound enzymes are less inhibited by glucose-1,6-diphosphate than the soluble forms, which is valid for both type I ($P < 0.001$) and type II ($P < 0.001$) hexokinase.

DISCUSSION

Increased glycolytic capacity is a well-known phenomenon of neoplastic tissues. The greater part of systematic studies on tumor glycolysis and on particular enzymes of tumor glycolysis were performed on rat hepatomas, because they provide a spectrum of well-defined tumors ranging from slowly growing, well-differentiated to rapidly proliferating, undifferentiated tumors. Hexokinase activity in these tumors in general correlated with growth rate and differentiation [3–5, 8, 9]. More precisely, tumor growth rate and glycolytic activity were shown to be correlated with the amount of mitochondrial-bound hexokinase. This tumor mitochondrial-bound hexokinase was directly coupled to oxidative phosphorylation: addition of glucose to respiring hepatoma mitochondria resulted in stimulation of respiration [3], whereas glucose had no effect on the respiration of mitochondria from control and regenerating liver, which bear no hexokinase on their membrane. Furthermore, addition of tumor mitochondria to normal rat liver cytosol produced a stimulatory effect on glycolysis, while tumor mitochondria devoid of hexokinase did not [4]. These observations supported strongly the hypothesis that the localization of hexokinase plays a key role in the increased glycolytic activity of neoplastic tissues.

In this study we tested this hypothesis in medullary thyroid carcinomas (MTC) from rats of the Wag/Ry strain. Some aging rats of this strain develop spontaneously slowly growing, well-differentiated MTC, secreting peptide hormones in large quantities. These tumors can be maintained by transplantation in other rats. After sequential transplantation some of these tumors showed an increased growth rate and became apparently more malignant. Only minor amounts of peptide hormones were excreted and these tumors were microscopically proven to be anaplastic (AMTC). These tumors are supposed to be derived from the thyroid parafollicular C cells and are used as a model for well-differentiated and undifferentiated tumors, respectively, of the APUD series (amine precursor uptake and decarboxylation) [18].

Despite the increase in lactic acid content in AMTC, indicative for an increased *in vivo* glycolytic activity, and despite a marked differ-

ence in growth rate, no difference was found in either hexokinase activity or localization. The major part of hexokinase in both tumors was soluble. Furthermore, the kinetic characteristics of hexokinase isoenzymes in both tumors were similar. The only difference between AMTC and DMTC was a slight increase in the proportion of hexokinase type II in line with several observations on other tumors [6, 7, 9, 19, 20]. However, this rather minor shift can hardly be expected to result in an increase in overall glycolytic activity. In conclusion, the results presented here suggest that the correlation between the level of mitochondrial bound hexokinase activity and tumor growth rate or glycolytic activity observed in hepatomas is not a general phenomenon of cancer tissues. In contrast, the amount of mitochondrial bound hexokinase in some human brain tumors, e.g. gliomas and astrocytomas, was decreased with respect to normal brain [Sprengers *et al.*, unpublished observations].

In our tumor system an *in vivo* increase of glycolytic rate in AMTC compared to DMTC should be provoked by other factors. The enzymes phosphofructokinase—a regulatory enzyme of glycolysis as well—and Na-K-ATPase are likely candidates. Regulation of phosphofructokinase was shown to be affected in three transplantable rat thyroid tumors to an extent which correlated with the growth rate of the tumors [21]. In other studies an ineffective Na-K-ATPase present in tumor cells was shown to provoke high glycolytic rates [22, 23].

The investigation of the kinetic properties of soluble and bound type I and II hexokinases produced some additional data: first, the affinities of the soluble and bound forms of both isoenzymes for their substrates glucose and $MgATP^{2-}$ were the same under the experimental conditions. In some studies an increased affinity of bound type I hexokinase for $MgATP^{2-}$ was reported compared to the soluble form. However, the conditions necessary to produce such a difference are still a matter of dispute [1]. Second, comparison of type I and II hexokinases shows that not only the affinity for the subtrate glucose is lower for the type II isozyme—a well-known fact from literature [24]—but that also the K_m $MgATP^{2-}$ is significantly higher. The latter probably accounts for the higher susceptibility of type II hexokinase for inhibition by glucose-1,6-diphosphate, as reported by Beitner [25]. Indeed, also in our experiments, at one fixed $MgATP^{2-}$ concentration type I hexokinase is less inhibited by glucose-1,6-diphosphate than type II. However, determination of true inhibition constants produces equal numbers for type I and II isoenzymes, as was shown in the present study. Third, the decreased inhibition by phosphorylated hexoses of bound hexokinase with respect to soluble hexokinase, which was well documented before for the type I isoenzyme, is to the same degree valid for type II hexokinase.

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REFERENCES

1. Wilson JE. Brain hexokinase, the prototype ambiquitous enzyme. In: Horecker BL, Stadtman ER, eds. *Current Topics in Cellular Regulation*. New York, Academic Press, 1980, Vol. 16, 1–45.
2. Felgner PL, Messer JL, Wilson JE. Purification of a hexokinase binding protein from the outer mitochondrial membrane. *J Biol Chem* 1979, **254**, 4946–4949.
3. Bustamante E, Pedersen PL. High aerobic glycolysis of rat hepatoma cells in culture: role of mitochondrial hexokinase. *Proc Natl Acad Sci USA* 1977, **74**, 3735–3739.
4. Bustamante E, Morris HP, Pedersen PL. Energy metabolism of tumor cells. Requirement for a form of hexokinase with a propensity for mitochondrial binding. *J Biol Chem* 1981, **256**, 8699–8704.
5. Bustamante E, Pedersen PL. Mitochondrial hexokinase of rat hepatoma cells in culture: solubilization and kinetic properties. *Biochemistry* 1980, **19**, 4972–4977.
6. Kikuchi Y, Sato S, Sugimura T. Hexokinase isozyme patterns of human uterine tumors. *Cancer* 1972, **30**, 444–447.
7. Bennett MJ, Timperley WR, Taylor CB, Hill AS. Isozymes of hexokinase in the developing, normal and neoplastic human brain. *Eur J Cancer* 1978, **14**, 189–193.
8. Shatton JB, Morris HP, Weinhouse S. Kinetic, electrophoretic, and chromatographic studies on glucose-ATP phosphotransferases in rat hepatomas. *Cancer Res* 1969, **29**, 1161–1172.
9. Hammond KD, Balinsky D. Isozyme studies of several enzymes of carbohydrate metabolism in human adult and fetal tissues, tumor tissues, and cell cultures. *Cancer Res* 1978, **38**, 1323–1328.

10. Kosow DP, Rose IA. Ascites tumor mitochondrial hexokinase II. Effect of binding on kinetic properties. *J Biol Chem* 1968, **243**, 3623–3630.
11. Katzen HM, Soderman DD, Wiley CE. Multiple forms of hexokinase. Activities associated with subcellular particulate and soluble fractions of normal and streptozotocin diabetic rat tissues. *J Biol Chem* 1970, **245**, 4081–4096.
12. Singer FR, Habener TJF. Multiple immunereactive forms of calcitonin in human plasma. *Biochem Biophys Res Commun* 1974, **61**, 710–716.
13. Lips CJM, van der Donk JA, Huber-Spanier R *et al.* The synthesis of calcitonin and β-endorphin by C cell-derived tumours; the possibility of a common precursor. In: Pecile A, ed. *Calcitonin* 1980. *International Congress Series No.* 540. Amsterdam, Exerpta Medica, 1980, 45–52.
14. Rijksen G, Staal GEJ. Purification and some properties of human erythrocyte hexokinase. *Biochim Biophys Acta* 1976, **445**, 330–341.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **139**, 265–275.
16. Cleland WW. Statistical analysis of enzyme kinetic data. *Methods Enzymol* 1979, **63A**, 102–128.
17. Kraaijenhagen RJ, Rijksen G, Staal GEJ. Hexokinase isozyme distribution and regulatory properties in lymphoid cells. *Biochim Biophys Acta* 1980, **631**, 402–411.
18. Pearse AGE. The APUD concept and hormone production. *Clin Endocrinol Metab* 1980, **9**, 211–222.
19. Sekiya S, Kikuchi Y, Takamizawa H. High- and low-tumorigenic culture lines of rat uterine adenocarcinoma and their isozyme patterns of lactate dehydrogenase and hexokinase. *Cancer Res* 1973, **33**, 3324–3329.
20. Kamel R, Schwarzfischer F. Hexokinase isozymes in human neoplastic and fetal tissues: the existence of hexokinase II in malignant tumors and in placenta. *Hum Genet* 1975, **30**, 181–185.
21. Meldolesi MF, Laccetti P. Phosphofructokinase regulation in some transplantable thyroid tumors. *Cancer Res* 1979, **39**, 2796–2801.
22. Racker E. *A New Look at Mechanisms in Bioenergetics.* New York, Academic Press, 1976, 163.
23. Racker E. Why do tumor cells have a high aerobic glycolysis? *J Cell Physiol* 1976, **89**, 697–700.
24. Pürich DL, Fromm HJ, Rudolph FB. The hexokinases: kinetic, physical and regulatory properties. *Adv Enzymol* 1973, **39**, 249–326.
25. Beitner R. The role of glucose-1,6-biphosphate in the regulation of carbohydrate metabolism in muscle. *Trends Biochem Sci* 1979, **4**, 228–230.