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Human Liver Glycogen Phosphorylase

KINETIC PROPERTIES AND ASSAY IN BIOPSY SPECIMENS

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1. The two forms of glycogen phosphorylase were purified from human liver, and some kinetic properties were examined in the direction of glycogen synthesis. The *b* form has a limited catalytic capacity, resembling that of the rabbit liver enzyme. It is characterized by a low affinity for glucose 1-phosphate, which is unaffected by AMP, and a low V, which becomes equal to that of the *a* form in the presence of the nucleotide. Lyotropic anions stimulate phosphorylase *b* and inhibit phosphorylase *a* by modifying the affinity for glucose 1-phosphate. Both enzyme forms are easily saturated with glycogen. 2. These kinetic properties have allowed us to design a simple assay method for total (*a*+*b*) phosphorylase in human liver. It requires only 0.5 mg of tissue, and its average efficiency is 90% when the enzyme is predominantly in the *b* form. 3. The assay of total phosphorylase allows the unequivocal diagnosis of hepatic glycogen-storage disease caused by phosphorylase deficiency. One patient with a complete deficiency is reported. 4. The assay of human liver phosphorylase *a* is based on the preferential inhibition of the *b* form by caffeine. The *a* form displays the same activity when measured by either of the two assays.

The diagnosis of liver phosphorylase deficiency in hepatic glycogenosis requires the determination of total (*a*+*b*) phosphorylase in the biopsy specimen, because the presence of the enzyme as the inactive *b* form could mimic the congenital deficiency; indeed, liver phosphorylase *b* has for long been considered as completely inactive (Appleman *et al.*, 1966), and the activity that it can display varies greatly from species to species and also according to the ionic conditions of the assay (Stalmans & Hers, 1975). A straightforward approach is to measure the activity of the *a* form after complete conversion of phosphorylase *b* into *a* by purified phosphorylase *b* kinase; the systematic application of this procedure is, however, precluded by the usual paucity of human liver biopsy material. Simple methods have been described for the specific determination of phosphorylase *a* and of total phosphorylase in rat liver (Stalmans & Hers, 1975; Tan & Nuttall, 1975). Phosphorylase *a* can be measured specifically in the presence of caffeine, which decreases the activity of the *b* form to a negligible value; assay of total phosphorylase is possible by inclusion of AMP and of lyotropic anions (i.e. anions that are high on the Hofmeister or lyotropic series), which render both enzyme forms equally active. Phosphorylase *b* can also be measured by greatly increasing the concentration of glucose 1-phosphate in the assay mixture.

The aim of the work described in the present paper was to check whether the above procedures could apply to human liver phosphorylase. For this purpose the two forms of the enzyme have been purified. In contrast with the rat or mouse enzyme, the activities of the *a* and *b* forms in the presence of AMP plus sulphate were still greatly different and could not be taken as a measure of the total (*a*+*b*) phosphorylase. It was therefore necessary to investigate the kinetic properties of phosphorylase *a* and *b* in more detail, in order to define conditions that would allow an appropriate assay of total phosphorylase of human liver samples.

Materials and Methods

Purification of human liver phosphorylase

b form. The liver of a 13-year-old girl, who died after a road accident, was obtained at autopsy performed 5 h after death. The liver was stored frozen at −20°C for about 1 month. Phosphorylase *b* was purified by a previously described method (Stalmans & Hers, 1975), with the following modifications: (*a*) 750 g of frozen tissue was pulverized by percussion in a mortar cooled with liquid N_2; the powder was homogenized for 1 min in a Waring Blendor with 6 vol. of 1 mM-EDTA, pH 7; (*b*) the DEAE-cellulose chromatography was performed by the application of a linear gradient (0–0.5 M) of NaCl dissolved in 1 mM-EDTA, pH 7. The enzyme was eluted at 0.25 M-

NaCl. The final recovery was 25% and the purification was 110-fold.

a form. Phosphorylase *a* was prepared by complete activation of the purified *b* enzyme by phosphorylase *b* kinase. For this purpose, phosphorylase *b* (0.7mg of protein) was incubated with 1.5mM-ATP, 2.5mM-magnesium acetate and 0.8 unit of muscle phosphorylase *b* kinase (free of phosphorylase *b*) in a total volume of 0.2ml for 30min at 30°C. Since the *a* form was assayed at a subsequent dilution of 400-fold, it was considered unnecessary to reisolate this enzyme from the activation mixture. The purified enzymes were diluted with a solution containing 10mM-glycylglycine and 0.1% serum albumin, adjusted to pH7.4.

Human liver specimens

The liver samples from patients D., X., F. G., R. K. and T. P. had been obtained at autopsy performed 1–8h after death, and had been stored at −20°C for periods ranging from 1 to 10 years. Death was due to trauma (D. and X.), type II glycogenosis (F. G.), or unestablished cause (R. K. and T. P.). The liver specimen from patient D. was badly preserved, since at the time of the present assays the activity of glucose 6-phosphatase was close to zero. Samples weighing about 1g were homogenized in a conical sintered-glass grinder (Kontes Glass Co., Vineland, NJ, U.S.A.; Duall 23) with 4vol. of an ice-cold solution containing 50mM-NaF and 10mM-glycylglycine, pH7.4.

Patient C. M. L. (from Professor R. Bernard, Marseille, France), a 17-month-old girl, suffered from retarded growth, hepatomegaly and hypoglycaemia. A surgical liver biopsy was taken, from which a fragment was sent to our laboratory; 12mg of frozen tissue was homogenized as above with 1.2ml of water. The analysis revealed an elevated glycogen content (65mg/g of tissue; 0.86mg/mg of protein); the activity of phosphorylase, determined by Hers' (1959) method, was zero. Normal values were found for the activities of glucose 6-phosphatase, amylo-1,6-glucosidase, acid α-glucosidase and phosphorylase *b* kinase.

Assay of phosphorylase

Phosphorylase was assayed in the direction of glycogen synthesis. The activity of the enzyme was measured by the incorporation of radioactivity from [^{14}C]glucose 1-phosphate into glycogen. This radiochemical assay was adopted instead of the usual colorimetric method (e.g. Hers, 1959), which measures the production of P_i, for two main reasons. First, we have in the course of this work been forced to increase greatly the concentration of glucose 1-phosphate in the assay mixture; consequently the blank values in the colorimetric assay became unacceptably high. Secondly, the occasional presence of fluoride at a concentration above 0.3M interfered with the correct development of the colour in the optical method.

In the standard assay, 0.15ml of reaction mixture contained 2mg of glycogen, 10μmol (0.03μCi) of [U-^{14}C]glucose 1-phosphate, 30μmol of NaF, and other additions as specified. The modified '*a* assay' mixture contained, in a volume of 0.15ml, 2mg of glycogen, 40μmol (0.03μCi) of [U-^{14}C]glucose 1-phosphate, 30μmol of NaF and 0.1μmol of caffeine. The modified '*a*+*b* assay' mixture contained, in 0.15ml, 2mg of glycogen, 40μmol of [U-^{14}C]-glucose 1-phosphate, 30μmol of NaF, 0.1mmol of $(NH_4)_2SO_4$ and 1μmol of AMP. All the assay mixtures were adjusted to pH6.5 with HCl.

The assays were performed as follows: 0.05ml of enzyme preparation (a 1% liver homogenate or suitably diluted purified enzyme) was added to 0.15ml of assay mixture and incubated at 30°C for various times, up to 30min. Four blank values were included in each experimental set by adding water instead of enzyme. At the end of the incubation, 0.15ml of the mixture was spotted on a square (3cm×3cm) of Whatman ET-31 filter paper, which was then dropped in ice-cold acid 66% (v/v) ethanol. The papers were then washed and counted for radioactivity as detailed by Gilboe *et al.* (1972), with the following modifications: (*a*) to increase the solubility of glucose 1-phosphate, 1% trichloroacetic acid was added to the first bath of ice-cold 66% ethanol; the papers were washed in this solution for 20min at 0°C; (*b*) the papers were then transferred to 66% ethanol at room temperature (20–25°C), and washed again for 20min; the latter procedure was repeated twice more; (*c*) the beakers contained at least 30ml of solution for each paper. These modifications eliminated the problem encountered by Gilboe *et al.* (1972), that high blank values occurred when more than 0.5mg of glycogen was spotted on each paper. With the modified '*a*+*b* assay' mixture (see above) the blank values (mean±S.E.M.) were 47±5c.p.m. (n=12), whereas after 30min of incubation with 0.05ml of 1% liver homogenates (Table 3) the values were 320±24c.p.m. (n=16). It has been verified that the counting efficiency of [^{14}C]glycogen (42000 c.p.m./mg) was independent of the amount of glycogen spotted, up to at least 4.5mg per paper square, i.e. 3 times the amount transferred during the regular assays. The conversion of the substrate into product was always kept below 5%, and the rate of the reaction was linear with respect to incubation time.

Activation of phosphorylase in liver homogenates

Full conversion of phosphorylase into the *a* form was achieved as follows: 0.4ml of homogenate was diluted to 0.5ml by the successive addition of muscle phosphorylase *b* kinase (0.8 unit) and 1μmol of ATP mixed with 1.5μmol of magnesium acetate. The

activation was complete after incubation for 30min at 30°C. Before assay the initial homogenate and the preincubated mixture were diluted 20-fold with a solution containing 15mM-NaF and 10mM-glycylglycine, pH7.4.

Miscellaneous

Glucose 1-phosphate was purchased from Fluka A.G., Buchs, Switzerland, and Pipes [piperazine-*NN'*-bis-(2-ethanesulphonic acid)] from Sigma Chemical Co., St. Louis, MO, U.S.A. Phosphorylase *b* kinase was purified from rabbit muscle as described by Brostrom *et al.* (1971) to the stage of precipitation with $(NH_4)_2SO_4$, and dialysed against a solution containing 2mM-EDTA and 50mM-sodium glycerol 2-phosphate, pH6.8. One unit of phosphorylase *b* kinase is the amount of enzyme that produces one unit of phosphorylase *a*/min in the colorimetric assay described by Lederer *et al.* (1975). The source of other materials has previously been reported, as well as methods that have not been described above (Stalmans *et al.*, 1974; Lederer *et al.*, 1975).

Results

General characteristics of the two enzyme forms

Table 1 shows a first approximation of the relative activities of human liver phosphorylases *b* and *a*; the purified enzymes were assayed under standard assay conditions with the indicated additions. In the presence of 0.5mM-caffeine the activity of the *b* form relative to the *a* form was negligible, and the assay is specific for the *a* form, as it is for all other mammalian species examined (Stalmans & Hers, 1975). With and without added AMP, the activity of phosphorylase *b* was considerably lower than that of the enzyme from rat or mouse liver (Stalmans & Hers, 1975). The *a* form was slightly stimulated by AMP and slightly inhibited by caffeine. However, in the presence of AMP plus sulphate the activity of the *b* form was only about half that of the *a* form; the simple procedure devised for the assay of total phosphorylase in rat and mouse liver (Stalmans & Hers, 1975) could therefore not be directly applied to human liver. This prompted us to investigate the kinetic properties of the enzyme, in order to define conditions that would allow an appropriate assay of total phosphorylase in human liver samples.

Table 1. *Activity of the two enzyme forms in standard assay conditions*

Initial reaction rates of purified phosphorylase *a* and *b* were measured in the presence of 1% glycogen, 50mM-glucose 1-phosphate, 0.15M-NaF, and other additions as indicated. Values shown are means of at least two separate determinations.

Additions	Activity (μmol/min per mg of protein) *a* form	*b* form	Activity ratio (*b* as % of *a*)
None	14.4	0.5	3.5
0.5mM-Caffeine	12.8	0.03	0.23
2mM-AMP	15.2	4.4	29
2mM-AMP plus 0.5M-Na_2SO_4	11.1	6.3	57

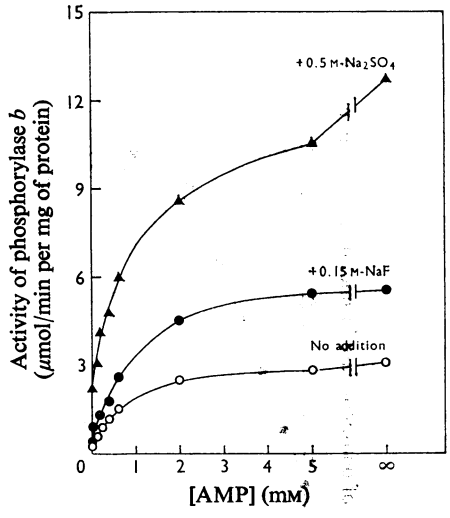

Fig. 1. *Effect of AMP on the activity of phosphorylase b*

Purified phosphorylase *b* was assayed in the presence of 1% glycogen, 50mM-glucose 1-phosphate, variable concentrations of AMP, and other additions as indicated. '∞ AMP' indicates the activities at infinite concentration of AMP, as estimated from double-reciprocal plots of the AMP effect.

Effect of AMP and of salts

Fig. 1 shows the saturation kinetics of phosphorylase *b* with AMP, and the effect of fluoride (0.15M) and of sulphate (0.5M) thereupon. The affinity of the enzyme for the nucleotide was unaffected by the presence of salt (apparent $K_a = 0.8$–1.2mM); fluoride and sulphate increased the activity of the enzyme both in the absence of AMP and at saturating concentrations of the nucleotide. In view of the rather low affinity of the *b* enzyme for AMP, the concentration of the nucleotide in further experiments was raised to 5mM.

Fig. 2 shows the effect of the concentration of two lyotropic anions on the activity of phosphorylase *b*. As for phosphorylases *b* from other mammalian species (Stalmans & Hers, 1975), these anions caused an extensive stimulation of enzyme activity, at least 8-fold in the absence of AMP and 3–4-fold in the

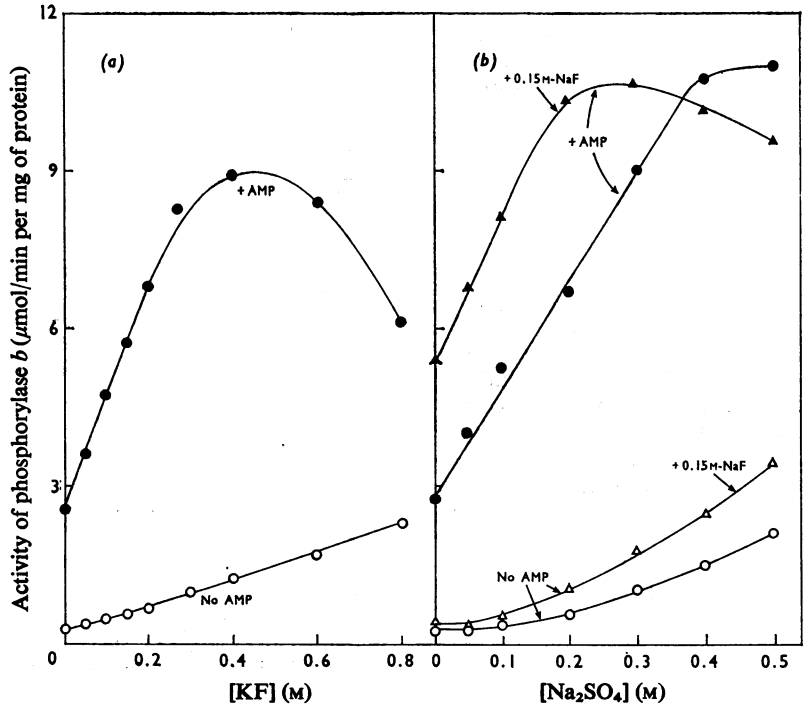

Fig. 2. *Effect of anions on the activity of phosphorylase b*

The purified *b* enzyme was assayed in the presence of 1% glycogen and 50mM-glucose 1-phosphate, without AMP (○, △) or with 5mM-AMP (●, ▲), and with the indicated concentrations of salts. In (*a*) the effect of increasing concentrations of KF is shown; in (*b*) increasing concentrations of Na_2SO_4 were added, without (○, ●) or with (△, ▲) 0.15M-NaF.

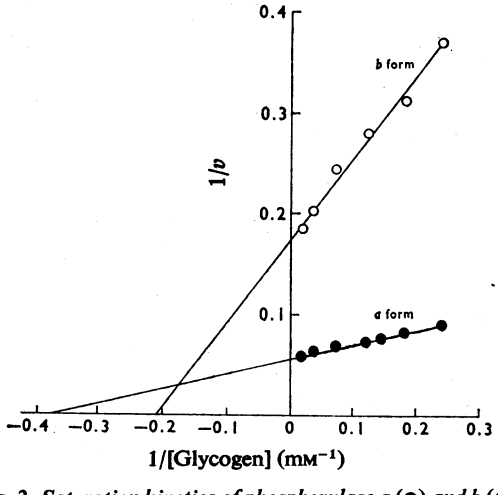

Fig. 3. *Saturation kinetics of phosphorylase a* (●) *and b* (○) *with glycogen*

The purified enzymes were incubated in the presence of 50mM-glucose 1-phosphate, 5mM-AMP, 0.15M-NaF and variable concentrations of glycogen, indicated as glucose equivalents. *v* is expressed as μmol/min per mg of protein.

presence of 5mM-AMP. Under the latter conditions, the optimal total salt concentration was around 0.5M. Below that concentration the effects of the two salts were additive. Sulphate was somewhat more potent than fluoride. KF and NaF behaved identically (not shown); the extent of stimulation was slightly smaller with Na_2SO_4 than with $(NH_4)_2SO_4$, which was also preferred in subsequent work because of its greater solubility.

Kinetics of saturation with substrates

Fig. 3 illustrates, in the form of a double-reciprocal plot, the saturation kinetics of both enzyme forms with glycogen in standard assay conditions plus 5mM-AMP. The apparent K_m values for glycogen, at 50mM-glucose 1-phosphate, were 2.7mM (*a* form) and 4.8mM (*b* form), expressed as glucose equivalents. The concentration of glycogen used previously in the standard assay (10mg/ml or 56mM) was already saturating and accordingly kept as such.

Table 2 summarizes the kinetic parameters of the two forms of phosphorylase with respect to glucose 1-phosphate. It appears that phosphorylase *b* is characterized by a very high K_m for glucose 1-

Table 2. *Kinetic parameters of phosphorylase with respect to glucose 1-phosphate*

Initial reaction rates of purified phosphorylase *a* and *b* were measured in the presence of 1% glycogen, variable concentrations of glucose 1-phosphate, 10mM-Pipes, pH6.5, and AMP and salts as indicated. Values shown are means of two separate determinations.

Enzyme form	Additions			K_m (glucose 1-phosphate) (mM)	V (μmol/min per mg of protein)
	5mM-AMP	0.15M-NaF	0.5M-$(NH_4)_2SO_4$		
b	−	+	−	120	1.7
	+	+	−	120	18.4
	+	+	+	59	17.8
a	−	−	−	2	15.5
	+	−	−	0.7	16.6
	−	+	−	9	18.2
	+	+	−	6	17.4
	+	+	+	20	18.4

Table 3. *Determination of phosphorylase a and a+b in human liver specimens*

Enzyme activities were measured with the modified assay mixtures (see the Materials and Methods section) and are expressed as μmol/min per mg of protein (purified enzyme) or as μmol/min per g of liver (crude homogenates). Initial reaction rates were determined with the purified enzymes and with a single homogenate of each autopsy sample. The activity of phosphorylase in the homogenized biopsy (C. M. L.) was determined in duplicate after 30min of incubation only. General data on the various liver samples are given in the Materials and Methods section.

	Activity before activation		Activity after activation		Activity ratio ('before' as % of 'after')	
	a assay	*a*+*b* assay	*a* assay	*a*+*b* assay	*a* assay	*a*+*b* assay
Purified enzyme	0.3	14.9	17.4	17.9	1.7	83
Autopsy samples						
X.*	0.1	18.0	19.6	21.3	0.5	85†
D.	1.4	13.1	17.7	16.5	8	80
F. G.	0.4	16.0	17.1	16.2	2.3	99
R. K.	0.6	13.7	14.8	14.8	4	93
T. P.	2.2	25.3	27.2	27.2	8	93
Mean ($n = 5$)	0.9	17.2	19.3	19.2	4.6	90
±S.E.M.	±0.4	±2.2	±2.1	±2.3	±1.5	±3.3
Biopsy of C. M. L.		0.0				

* Liver from which the enzyme was purified.
† With the assay method of Hers (1959) this value was 25%.

phosphate, when measured in the presence as well as in the absence of AMP. Indeed, the stimulatory effect of the nucleotide on the *b* form was entirely due to an increased V, which, in the presence of AMP, became equal to that of phosphorylase *a*. The addition of 0.5M-$(NH_4)_2SO_4$ increased twofold the affinity of phosphorylase *b* for glucose 1-phosphate without changing V. AMP had little effect on V of phosphorylase *a*, but it lowered somewhat the K_m value for glucose 1-phosphate. This K_m value was decreased in the absence of fluoride and increased when sulphate was added.

It appears from these kinetic data that the activity of both enzyme forms should be approximately equal when substrates and AMP are present at sufficiently high concentrations. However, the limited solubility of glucose 1-phosphate renders it technically impossible to achieve such assay conditions. We have therefore increased the concentration of the latter substrate to 0.2M, the highest practicable value.

Modified assay of phosphorylase a and of total phosphorylase

As a result of the kinetic analysis of the two forms, we chose to assay the enzyme in the presence of 0.2M-glucose 1-phosphate, 1% glycogen and 0.15M-NaF. The addition of caffeine (0.5mM) inhibits the *b* form (Table 1), and renders the assay specific for the *a* form. For the determination of total phosphorylase, caffeine is omitted but 5mM-AMP and 0.5M-

$(NH_4)_2SO_4$ are added; one can calculate from Table 2 that the activity of the *b* form should then reach about 85% of that of the *a* form. The exact composition of the modified assay mixtures is listed in the Materials and Methods section.

Table 3 shows the results of these two assays with the purified *a* and *b* forms, and with crude homogenates of frozen post-mortem liver samples where sufficient material was available to analyse the enzyme before and after full conversion into the *a* form. With either type of assay, phosphorylase *a* displayed the same activity. The assay for total phosphorylase yielded a 15% lower value for the purified *b* form than for the *a* enzyme; identical results were obtained with a homogenate of the liver from which the enzyme had been purified. In the post-mortem liver samples, total phosphorylase was determined with an efficiency ranging from 80 to 99%. Before activation less than 10% of the enzyme in the liver homogenates was in the *a* form; values below 2% should not be considered different from zero, since the *b* enzyme has some activity in the '*a* form assay.'

Also included in Table 3 is the result of an analysis of the biopsy specimen from C. M. L., an infant suspected of hepatic phosphorylase deficiency. No trace of enzyme activity was found with the new '*a*+*b* assay'.

Discussion

Characteristics of human liver phosphorylase

The kinetic parameters of human liver phosphorylases *a* and *b* are to a large extent in good agreement with published values for the liver enzymes from other mammalian species. The affinity of the *b* form for AMP is similar to that of the enzymes from rabbit and rat (Stalmans & Hers, 1975; Tan & Nuttall, 1975). The same can be said of the apparent K_m values of the two forms for glycogen (Maddaiah & Madsen, 1966; Tan & Nuttall, 1975). The affinity of the *a* form for glucose 1-phosphate is again close to that reported for the *a* forms from rabbit and dog in comparable assay conditions (Maddaiah & Madsen, 1966; Stalmans *et al.*, 1974); it was, however, sharply lowered by the addition of fluoride or sulphate. As to the effect of AMP on the saturation kinetics of the *b* form with glucose 1-phosphate, a striking difference exists between our data on the human enzyme and those of Tan & Nuttall (1975) on the rat enzyme. AMP increased V of the human enzyme 10-fold, without affecting the low affinity for glucose 1-phosphate; with the rat enzyme, the affinity increased and V remained unchanged and equal to that of the *a* form. Further, AMP induced a co-operative binding of glucose 1-phosphate by the rat enzyme; this puzzling feature was not encountered with the human enzyme, which followed Michaelis–Menten kinetics in all conditions examined.

We have previously examined the properties of liver phosphorylases *b* from several mammalian species; it appeared that the activities of these enzymes, relative to the *a* forms, were intrinsically different, being highest in rat and mouse, lower in the rabbit, and very low in the pig (Stalmans & Hers, 1975). Phosphorylase *b* from human liver appears to be closely related to the rabbit liver enzyme, and the responses of these enzymes to salts are strikingly similar. It may be remembered that glycogen phosphorylases from rabbit and human skeletal muscle, as judged by their immunological and physicochemical properties, are also closely related (Yunis *et al.*, 1960; Hughes *et al.*, 1962), though not identical (Yunis & Krebs, 1962).

It is noteworthy that human liver phosphorylase appears to be distinctly different from the enzyme present in human haemolysates. In the latter case both enzyme forms in the absence or presence of AMP display the same K_m for glucose 1-phosphate; AMP and interconversion between the two forms affect V exclusively (Lederer *et al.*, 1975). This difference indicates that the activity of phosphorylase in blood samples is poorly representative of the liver enzyme.

Diagnosis of liver phosphorylase deficiency

Hers (1959) reported three children with hepatic glycogen-storage disease, characterized by normal activities of glucose 6-phosphatase and amylo-1,6-glucosidase in their livers, but an activity of glycogen phosphorylase that was only 20–25% of the average normal value. It was stated that the complexity of the phosphorylase system did not allow a conclusion as to the exact nature of the enzymic defect responsible for the disease. Further, the examination of larger series of patients with hepatomegalic glycogen-storage disease not belonging to types I, II or III revealed that all intermediary values between normal and very low activities of liver phosphorylase could be found, even among affected sibs (Hers, 1961; Illingworth, 1961). With the same assay method (Hers, 1959) a few more similar patients have since been described (Drummond *et al.*, 1970; Spencer-Peet *et al.*, 1971; Guibaud & Mathieu, 1972); their activity of liver phosphorylase was 5–25% of the mean normal value, and the disease was attributed to a primary defect in liver phosphorylase. However, one obvious possibility is that phosphorylase was not really deficient, but that an unusually low estimate of phosphorylase activity is obtained when most or all of the enzyme is present in the *b* form; the latter situation can be the result of a phosphorylase *b* kinase deficiency, but it was also the rule rather than the exception in the post-mortem liver samples in our control series (Table 3).

The present work shows that, according to the precise assay conditions, human liver phosphorylase *b* can display an activity between zero and 90% of the activity of the *a* form; with the assay of Hers (1959) this value is 25%. Fernandes *et al.* (1974) measured phosphorylase in the direction of glycogen degradation and found a complete lack of activity in liver biopsies from two brothers. However, this type of assay, conducted at low ionic strength, yielded essentially no activity with rabbit liver phosphorylase *b* even in the presence of 1mM-AMP (Stalmans, 1976). In some of the above reports the patients' leucocytes were found to have a lower phosphorylase activity as well. The heterogeneity of the blood leucocytes fraction does not facilitate the interpretation of the results; the diagnosis of liver phosphorylase deficiency should certainly not be based on the analysis of leucocytes only.

Evidently the unequivocal diagnosis of hepatic glycogen-storage disease caused by liver phosphorylase deficiency requires the determination of total (*a*+*b*) liver phosphorylase. One adequate procedure is to use purified phosphorylase *b* kinase in order to obtain a complete activation of phosphorylase before the assay of the *a* form. This method has allowed recognition of two patients whose total liver phosphorylase activity was 10% of normal (Hug *et al.*, 1974). However, it cannot be applied systematically to needle-biopsy specimens, which usually weigh about 10mg and must be used for a number of other determinations as well. The alternative method that we have elaborated for the assay of total (*a*+*b*) liver phosphorylase requires only 0.5mg of tissue. The assay is performed in the presence of 1% glycogen, 200mM-glucose 1-phosphate, 0.15M-NaF, 0.5M-$(NH_4)_2SO_4$ and 5mM-AMP. In the presence of AMP, *V* of the two enzyme forms is approximately equal; an elevated concentration of glucose 1-phosphate was adopted because of the high K_m of the *b* form for this substrate; the latter parameter in turn was lowered by the addition of lyotropic anions.

The activity that is measured when phosphorylase is completely in the *b* form is 90% of that when all the enzyme is in the *a* form. The assay was satisfactory even in one liver sample that was badly preserved, as indicated by a loss of glucose 6-phosphatase activity (Hers & Van Hoof, 1966). It has allowed us to detect one patient (C. M. L.) who appears to be completely deficient in liver phosphorylase.

If this appears desirable, the activity of phosphorylase *a* can be measured as well; the assay requires an additional 0.5mg of liver. It is performed in the presence of 1% glycogen, 200mM-glucose 1-phosphate, 0.15M-NaF and 0.5mM-caffeine; the addition of caffeine renders the assay specific for the *a* form. The measured activity can be directly compared with the (*a*+*b*) activity in order to obtain the amount of *a* enzyme as a percentage of total phosphorylase.

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References

Appleman, M. M., Krebs, E. G. & Fischer, E. H. (1966) *Biochemistry* **5**, 2101–2107
Brostrom, C. O., Hunkeler, F. L. & Krebs, E. G. (1971) *J. Biol. Chem.* **246**, 1961–1967
Drummond, G. I., Hardwick, D. F. & Israels, S. (1970) *Can. Med. Assoc. J.* **102**, 740–742
Fernandes, J., Koster, J. F., Grose, W. F. A. & Sorgedrager, N. (1974) *Arch. Dis. Child.* **49**, 186–191
Gilboe, D. P., Larson, K. L. & Nuttall, F. Q. (1972) *Anal. Biochem.* **47**, 20–27
Guibaud, P. & Mathieu, M. (1972) *Arch. Fr. Pediatr.* **29**, 1043–1057
Hers, H. G. (1959) *Rev. Int. Hepatol.* **9**, 35–55
Hers, H. G. (1961) *Chem. Weekbl.* **57**, 437–440
Hers, H. G. & Van Hoof, F. (1966) *Methods Enzymol.* **8**, 525–532
Hug, G., Chuck, G., Walling, L. & Schubert, W. K. (1974) *J. Lab. Clin. Med.* **84**, 26–35
Hughes, R. C., Yunis, A. A., Krebs, E. G. & Fischer, E. H. (1962) *J. Biol. Chem.* **237**, 40–43
Illingworth, B. (1961) *Am. J. Clin. Nutr.* **9**, 683–690
Lederer, B., Van Hoof, F., Van den Berghe, G. & Hers, H. G. (1975) *Biochem. J.* **147**, 23–35
Maddaiah, V. T. & Madsen, N. B. (1966) *J. Biol. Chem.* **241**, 3873–3881
Spencer-Peet, J., Norman, M. E., Lake, B. D., McNamara, J. & Patrick, A. D. (1971) *Q. J. Med.* **40**, 95–114
Stalmans, W. (1976) *Curr. Top. Cell. Regul.* **11**, 51–97
Stalmans, W. & Hers, H. G. (1975) *Eur. J. Biochem.* **54**, 341–350
Stalmans, W., Laloux, M. & Hers, H. G. (1974) *Eur. J. Biochem.* **49**, 415–427
Tan, A. W. H. & Nuttall, F. Q. (1975) *Biochim. Biophys. Acta* **410**, 45–60
Yunis, A. A. & Krebs, E. G. (1962) *J. Biol. Chem.* **237**, 34–39
Yunis, A. A., Fischer, E. H. & Krebs, E. G. (1960) *J. Biol. Chem.* **235**, 3163–3168