Diabetologia 9, 30—33 (1973)

Comparative Study of Enzyme Activities Degrading Sorbitol, Ribitol, Xylitol and Gluconate in Guinea Pig Tissues

H. Birnesser, H. Reinauer and S. Hollmann

Institute of Physiological Chemistry and Diabetes Research Institute at the University of Düsseldorf, Germany (F.R.G.)

Received: July 1, 1972, accepted: October 19, 1972

Summary. In guinea pig tissues the activities of the enzymes D-gluconokinase, sorbitol dehydrogenase, D-ribulo- and D-xylulokinase were measured. D-ribulose and D-xylulose were prepared by isomerisation of D-ribose and D-xylose in pyridine and separated by preparative paper chromatography. The activity patterns of the pentulokinases were identical in all tested organs. The highest activities of these two enzymes were found in adipose tissue, when referred to soluble cell protein, and was higher than the activity in liver and kidney. The high enzyme activities of the pentulokinases in adipose tissue may explain the antilipolytic effect of these pentitols and pentoses in diabetes. The activities of sorbitol dehydrogenase and gluconokinase showed a similar activity pattern in all tested organs of the guinea pig. The highest activities were found in liver and kidney and the lowest in the adipose tissue. The direct metabolism of gluconate in adipose tissue seems impossible. The activity of the pentulokinases are diminished in the tissues of the diabetic rat.

Key words: Activity of pentulokinases, sorbitol dehydrogenase and gluconokinase-organs of guinea pigs and of normal and diabetic rats.

I. Introduction

Pentoses and pentitols, which are metabolised without insulin by the pentose phosphate cycle and glucuronic acid pathway, had an important position in the dietetic treatment of diabetes mellitus. In the diabetes diet these pentoses and pentitols were used as "exchange carbohydrates". The "exchange carbohydrates" have diminished in importance in the dietetic treatment of diabetes mellitus because of their calory-content. Nevertheless, the special metabolism of these compounds, which have been shown to have an antilipolytic and antiketogenic effect, has clinical and biological importance.

The aim of the following paper concerns the activity pattern of the enzymes which catalyse the first steps in the degradation of xylitol, ribitol, D-ribulose, D-gluconic acid and sorbitol. Therefore, the activities of the following kinases and of a dehydrogenase have been determined in different organs of guinea pigs and rats: ATP: D-xylulose 5-phosphotransferase (EC 2.7.1.17) = D-xylulokinase, ATP: D-ribulose 5-phosphotransferase (EC 2.7.1.47) = D-ribulokinase, ATP: D-gluconate 6-phosphotransferase (EC 2.7.1.12) = D-gluconokinase, L-iditol: NAD oxidoreductase (EC 1.1.1.14) = sorbitol dehydrogenase.

II. Method

1. *Reagents*

a) From Boehringer, Mannheim: adenosine-5-triphosphate, sodium salt, nicotinamide-adenine-dinucleotide-phosphate, sodium salt, reduced nicotinamide-adenine-dinucleotide, sodium salt, 6-phosphogluconate-dehydrogenase from baker's yeast.

b) Maleinic acid anhydride and D-ribose from Theodor Schuchardt, München.

c) Dowex I X—4, 200—400 mesh, Cl^--Form, Dowex WX-50, H^+-Form from Serva-Entwicklungslabor, Heidelberg.

All other reagents were of analytical grade.

2. Preparation of D-ribulose and D-xylulose was performed according to Touster's method [3].

3. *Experimental animals*

The following experimental animals were used:

I. Male guinea pigs weighing 250—300 g from Firma Gierlich, Bochum,

II. male Wistar II rats (Firma Brünger, Bokel, Westfalen) with an average weight of 200 g.

Rats were made diabetic by intraperitoneal injection of alloxan [8].

The blood sugar concentration in the diabetic animals was analysed by the glucose-oxidase method. The glucose concentration in the urine was determined by test tape.

The animals were decapitated and heart, liver, kidney, spleen, muscle, brain and epididymal fat pad were excised as rapidly as possible, cooled immediately and then homogenized in 0.154 M KCl — 0.04 M $NaHCO_3$, pH = 7.2. The homogenization was performed at 0°C during 90 sec with a homogenizer of Firma Bühler, Erlangen. The blender was driven at top speed for 90 sec. The tissue homogenate was spun down at 100000 g for 30 min at 0°C. The clear supernatant was taken for the determination of enzyme activity and protein content, according to Lowry *et al.* [9].

The activity of the gluconokinase was measured according to Leder [10], of the sorbitol dehydrogenase according to Gerlach [11], of the D-ribulokinase and D-xylulokinase according to Hickman and Ashwell [12].

III. Results

1. Preparation of D-ribulose

a) Preparation of D-ribulose by paper chromatography (Touster [3])

D-ribulose was prepared from D-ribose after isomerisation in pyridine. The separation of D-ribulose from D-ribose was performed by paper chromatogra-

phy. The gain of D-ribulose was about 100 mg per paper sheet. The D-ribulose was eluted and concentrated to a colourless syrup. In the orcin reaction the characteristic absorption spectrum was seen with a maximum at 435, 540 and 670 nm. The ratios of the maximum absorption were $E_{435}/E_{540}=0.58$ and $E_{540}/E_{670}=0.68$. The purity of the isolated ribulose was tested by thin-layer chromatography on Kieselgel G. with the solvent system ethylacetic acid: isopropanol 65:35. The following R_f-values were found:

D-ribulose: 0.36, D-ribose: 0.26.

The preparation of D-xylulose was performed similarly. There was no D-ribose and D-xylose in our preparation of D-ribulose and D-xylulose as tested by thin-layer chromatography.

b) Preparation of D-ribulose and D-xylulose by ion exchange chromatography (Khym and Zill [7])

With this method we could not get satisfactory results. Although the elution buffer was varied over a wide range we could not find any pure fraction with the typical spectrum of the ribulose, the maximum absorption at 540 nm being always lower than at 435 nm. There were always impurities of D-ribose and D-xylose in our preparation as revealed by thin-layer chromatography.

b) Activity of the sorbitol dehydrogenase (Table 1)

Activity of sorbitol dehydrogenase was found in all investigated tissues. Like gluconokinase, the highest activity was found in liver and kidney. The testis had only 1/10 of this activity, followed by the spleen and the heart. In brain, muscle and adipose tissue there was only 0.5—3% of the activity found in liver and kidney.

c) Activity of the D-xylulokinase (Table 1)

The activity of this enzyme was found in all tested organs and was highest in the liver, when referred to wet weight. 25—30% of the activity in liver was found in kidney, muscle, adipose tissue, spleen and brain. In testis and heart there was only 15% of the liver activity.

When the activity of the enzyme was referred to soluble protein, the highest activity was found in adipose tissue, which exceeded the activity in liver by 160% (1 g adipose tissue contained 1.5% protein).

d) Activity of the D-ribulokinase (Table 1)

The highest activity was found in the liver, when referred to wet weight. Lower activities were found in spleen, kidney, muscle, adipose tissue, heart and testis (50—20%). The lowest enzyme activity, with 10% of

Table 1. *Activity of D-gluconokinase, D-xylulokinase, D-ribulokinase and sorbitol dehydrogenase in the organs of guinea pigs ($n=6$). Upper line: n moles/g wet weight × min. Lower line: n moles/mg protein × min*

Organs	D-gluconokinase	D-xylulokinase	D-ribulokinase	Sorbitol dehydrogenase
liver	378.3 ± 57.1	3710 ± 1080	2930 ± 540	5058 ± 793
	4.7 ± 0.9	53 ± 14.8	36.4 ± 6.3	64.1 ± 17.6
kidney	380 ± 53.53	1260 ± 126	1002 ± 430	4421 ± 1189
	6.7 ± 1.3	19.9 ± 1.4	14.9 ± 5.6	74.8 ± 18.2
heart	7.2 ± 1.4	435.6 ± 90.6	738 ± 231	317.8 ± 55.4
	0.15 ± 0.04	10.0 ± 2.7	17.1 ± 6.2	6.1 ± 1.0
adipose	n.m. ±	1019 ± 398	739.8 ± 251.7	29.1 ± 11.6
tissue	n.m. ±	86.3 ± 31.2	53.26 ± 17.7	1.94 ± 1.0
spleen	13.8 ± 3.2	893 ± 243	1099 ± 241	493 ± 227
	0.21 ± 0.03	12.9 ± 4.6	16 ± 5.73	6.36 ± 2.44
muscle	1.6 ± 0.61	1151.4 ± 601	976.1 ± 157.3	87.6 ± 8.1
	0.04 ± 0.02	17.06 ± 6.71	20.47 ± 4.03	2.34 ± 0.42
brain	7.5 ± 1.43	877 ± 144	288 ± 85	143.9 ± 28.1
	0.22 ± 0.02	29.7 ± 5.9	11.4 ± 3.8	4.26 ± 0.97
testis	37 ± 14.2	524 ± 169	523.8 ± 64.2	545 ± 200
	0.83 ± 0.18	12.8 ± 3.6	13.6 ± 1.3	13.1 ± 2.6

2. Determination of enzyme activity in the organs of the guinea pig

a) Activity of the gluconokinase (Table 1)

The activity of the gluconokinase was found in all tissues except adipose tissue, where the activity of the enzyme could not be determined. The highest activity was found in liver and kidney, when expressed in terms of wet weight as well as mg protein. The testis had only 10% of the activity compared with the liver. The other organs had only 0.5—4% of the activity found in liver.

the activity in liver, was found in the brain. When the enzyme activity was referred to soluble cell protein, adipose tissue exceeded the activity in the other organs (150%).

3. Comparison of the enzyme activities in the tested organs (Table 1)

Quantitative ratios of the single enzyme activities did not give constant proportions. The gluconokinase had the lowest activity of all ATP-transferases as seen in the 8 investigated organs, the activity being

only 1/10 of the D-xylulokinase and D-ribulokinase. The activities of ATP-transferases and sorbitol dehydrogenase have the same order of magnitude in the liver, kidney, heart, spleen, and testis. In adipose tissue the activity of the sorbitol dehydrogenase is only 3.5% of the D-xylulokinase and D-ribulokinase.

4. Activities of the gluconokinase and xylulokinase in normal and diabetic rats (Table 2)

The activities of the tested ATP-transferases are reduced by 50% in the organs of diabetic animals compared with the controls. The difference is statistically significant (for the xylulokinase $P < 0.01$ and for the gluconokinase $P < 0.005$).

Table 2. *Activity of D-xylulokinase in the epididymal fat pad of the normal and diabetic rat*

	n	n moles/g wet weight × min	n moles/mg protein × min
normal rats	7	485 ±192	55 ±18
diabetic rats	7	243.2± 59.1	27.3± 4.2
Activity of D-gluconokinase in the liver of normal and diabetic rats			
normal rats	6	237.1± 59.5	2.4± 0.6
diabetic rats	6	140.4± 30.2	1.4± 0.2

IV. Discussion

In recent years xylitol has attained some clinical application in diabetes therapy. The adult organism can degrade 10—20 mg xylitol per minute per kg body weight. This degradation can be compared with the metabolism of fructose and corresponds to approximately 80% of the glucose metabolism. Xylitol can be metabolised without insulin and it was assumed that approximately 80% of the xylitol is degraded in the liver, the remaining 20% being metabolised in extrahepatic organs, namely in the kidney [25]. The erythrocytes [17], the heart muscle of the guinea pig [18] and the adipose tissue [19, 20] are able to metabolise xylitol, too. In adipose tissue xylitol has an antilipolytic action without raising the blood sugar concentration; the radioactivity of the applied xylitol-^{14}C is found firstly as $^{14}CO_2$ or in lipids respectively [26]. As the metabolism of xylitol follows initial dehydrogenation with the aid of NAD-xylitol dehydrogenase (polyol dehydrogenase) via D-xylulose-5-phosphate, the activity of these enzymes was determined in several organs. Simultaneously the activities of the D-ribulokinase and D-gluconokinase were measured.

Surprisingly we found high enzyme activities of the pentulokinases in adipose and muscle tissues. The predominance of adipose tissue in the enzyme activities of the pentulokinases is seen when the enzyme activity is referred to soluble cell protein. For the metabolism of pentitols in adipose tissue free permeability of the polyalcohols across the cell membrane and dehydrogenation from pentitols to pentuloses must proceed. The permeation of the polyalcohols is not controlled by insulin [23]. The dehydrogenation of xylitol is performed by a NAD-xylitol dehydrogenase in adipose tissue [21]. The antiketogenic effect of xylitol in diabetes may be explained — apart from the effect of xylitol on insulin secretion in the islets of pancreas — by the metabolism in the liver and in adipose tissue, via xylulose-5-phosphate to CO_2, acetyl-CoA and α-glycerophosphate, respectively. These compounds are metabolites of the synthesis of fatty acids and triglycerides. The muscle tissue has high enzyme activities too. If these enzyme activities are referred to the whole muscle mass and the whole adipose tissue of the body, then the total activity in these tissues exceeds the activity of the liver and kidney. Therefore balances in the degradation of xylitol or ribitol must take into consideration the whole fat and muscle tissue of the organism.

The rate-limiting step in the degradation of xylitol seems to be the NAD-xylitol-dehydrogenase, the activity of this enzyme being lower in the fat pad than the activity D-xylulokinase (D-xylulokinase 55 ± 18 nMol/mg protein/min, NAD-xylitol-dehydrogenase 15.2 ± 3.4 nMol/mg protein/min [21]). In diabetic rats the activity of the gluconic acid pathway should be enhanced [22]. In contrast we found the activity of the D-xylulokinase diminished. In this field further investigations must be carried out.

In contrast to the pentulokinases the other two tested enzymes, D-gluconokinase and sorbitol dehydrogenase, show another activity pattern in the tested organs. We found low, sometimes not determinable activity of the D-gluconokinase in adipose and muscle tissue, so that the metabolism of D-gluconate seems only to be possible in liver, kidney, brain and heart muscle. In this case the liver and kidney must have a nutritive function for the adipose and muscle tissue.

Literatur

1. Hollmann, S.: Extraglycolytische Umsetzung. In: D-Glucose und verwandte Verbindungen in Medizin und Biologie. Hrsg. von Bartelheimer, H., Heyde, W., Thorn, W., p. 340. Stuttgart: Ferdinand Enke 1966.
2. Fromm, H.J.: D-Ribulokinase from aerobacter aerogenes. J. biol. Chem. **234**, 3097 (1959).
3. Touster, O.: D- and L-threo-pentulose (D- and L-xylulose). In: Methods in carbohydrate chemistry. Hrsg. von Whistler, R.L., Wolfrom, M.L., Band I, p. 98. New York and London: Academic Press, 1962.
4. Hollmann, S.: Nichtglycolytische Stoffwechselwege der Glucose. Stuttgart: Georg Thieme 1961.
5. Stahl, E.: Dünnschichtchromatographie. 2. Aufl., p. 794. Berlin-Heidelberg-New York: Springer 1967.
6. Putman, E.W.: Methods in Enzymology. Hrsg. von Colowick, S.P., Kaplan, N.O., Band 3, p. 57. New York: Academic Press 1957.

6a. Hochster, R.M.: The formation of phosphorylated sugars from D-xylose by extracts of pseudomonas hydrophila. Can. J. Microbiol. **1**, 346 (1955).

7. Khym, J.X., Zill, L.P.: The separation of sugars by ion exchange. J. Amer. chem. Soc. **74**, 2090 (1952).
8. Wieland, O., Löffler, G., Weiss, U., Neufeldt, J.: Zur Acetessigsäure- und Cholesterinbildung bei experimenteller Ketose. Biochem. Z. **333**, 10 (1960).
9. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folinphenol-reagent. J. biol. Chem. **193**, 265 (1951).
10. Leder, J.G.: Hog kidney gluconokinase. J. biol. Chem. **225**, 125 (1957).
Ballard, F.J., Oliver, I.T.: The effect of concentration on glucose phosphorylation and incorporation into glycogen in the livers of foetal and adult rats and sheep. Biochem. J. **92**, 131 (1964).
11. Gerlach, U.: Sorbit-Dehydrogenase. In: Methoden der enzymatischen Analyse. Hrsg. von H.-U. Bergmeyer. Weinheim/Bergstraße: Chemie GmbH 1962.
12. Hickman, J., Ashwell, G.: Purification and properties of D-xylulokinase in liver. J. biol. Chem. **232**, 737 (1958).
13. Dische, Z., Borenfreund, E.: A new spectrophotometric method for the detection and determination of keto sugars and trioses. J. biol. Chem. **192**, 583 (1951).
14. Kameyama, T., Shimazono, N.: Enzymic phosphorylation of D-ribulose in guinea-pig liver. Biochim. biophys. Acta **64**, 180 (1962).
15. Lang, K.: Utilisation of xylitol in animals and man. International symposion on metabolism, physiology and clinical use of pentoses and pentitols, Hakone, August 1967. Hrsg. von Horecker, B.L., Lang, K. Berlin-Heidelberg-New York: Springer 1969.
16. Bässler, K.H.: Adaptive processes concerned with absorption and metabolism of xylitol. International symposium on metabolism, physiology and clinical use of pentoses and pentitols, Hakone, August 1967. Hrsg. von Horecker, B.L., Lang, K. Berlin-Heidelberg-New York: Springer 1969.
17. Bässler, K.H., Reimold, W.V.: Lactatbildung aus Zuckern und Zuckeralkoholen in Erythrocyten. Klin. Wschr. **43**, 169 (1965).
18. Bickel, H.: Experimental findings on the utilization of xylitol in heart muscle of the guinea pig. International symposium on metabolism, physiology and clinical use of pentoses and pentitols, Hakone, August 1967. Hrsg. von Horecker, B.L., Lang, K. Berlin-Heidelberg-New York: Springer 1969.
19. Opitz, K.: The influence of xylitol and other polyols and sugars in its effect on fat mobilisation. International symposium on metabolism, physiology and clinical use of pentoses and pentitols, Hakone, August 1967. Hrsg. von Horecker, B.L., Lang, K. Berlin-Heidelberg-New York: Springer 1969.
20. Yamagata, S., Goto, Y., Ohneda, A., Anzai, M., Kawashima, S., Chiba, M., Maruhama, Y., Yamauchi, T.: Clinical effects of xylitol on carbohydrate and lipid metabolism in diabetes. Lancet **1965 II**, 918.
21. Petrich, Ch., Reinauer, H., Hollmann, S.: Vergleichende Aktivitätsmessungen der Enzyme des Glucuronsäure-Xylulose-Cyclus in der Leber, dem epididymalen Fettgewebe der Ratte. Z. klin. Chemie, **10**, 355 (1972).
22. Winegrad, A.J.: The activity of the glucuronic acid pathway in human and experimental diabetes. International symposion on metabolism, physiology and clinical use of pentoses and pentitols, Hakone, August 1967. Hrsg. von Horecker, B.L., Lang, K. Berlin-Heidelberg-New York: Springer 1969.
23. Bässler, K.H., Prellwitz, W.: Insulin und der Verteilungsraum von Xylit in eviscerierten Ratten. Klin. Wschr. **42**, 94 (1964).
24. Bässler, K.H., Prellwitz, W., Unbehaun, V., Lang, K.: Zur Frage der Eignung von Xylit als Zuckerersatz beim Diabetiker. Klin. Wschr. **40**, 791 (1962).
25. Schmidt, B., Fingerhut, M., Lang, K.: Über den Stoffwechsel von radioaktiv markiertem Xylit bei der Ratte. Klin. Wschr. **42**, 1073 (1964).
26. Mehnert, H., Summa, J.D., Förster, H.: Untersuchungen zum Xylitstoffwechsel bei gesunden, leberkranken und diabetischen Personen. Klin. Wschr. **42**, 383 (1964).
27. Wang, M.C., Meng, H.C.: Xylitol metabolism in extrahepatic tissues. Z. Ernährungswissenschaft, Suppl. **11**, 8 (1971).
28. Hollmann, S., Reinauer, H.: Stoffwechsel der Pentosen und Pentitole. Z. Ernährungswissenschaft, Suppl. **11**, 1 (1971).

Priv. Doz. Dr. H. Reinauer
Diabetes-Forschungsinstitut an der Universität
D-4000 Düsseldorf-Benrath
Hospitalstr. 1
Federal Republic of Germany