ON THE BIOCHEMICAL BASIS OF HEREDITARY FRUCTOSE INTOLERANCE

J.F. Koster[a], R.G. Slee[a] and J. Fernandes[b]

[a]Department of Biochemistry I, [b]Department of Pediatrics, Medical Faculty, Erasmus University Rotterdam (The Netherlands)

Received March 18,1975

SUMMARY. In hereditary fructose intolerance it was found that in addition to an increased K_m value for Fru-1-P, the K_m of aldolase for Fru-1,6-P_2 was also increased. Furthermore, human phosphorylase *a* was found to be inhibited by Fru-1-P in a non-competitive way.

Human hereditary fructose intolerance (HFI) is due to a deficiency of Fru-1-P aldolase (liver type aldolase or aldolase B) (EC 4.1.2.7) (ref. 1). It has been stated that the aldolase activity with Fru-1,6-P_2 as substrate is diminished. This, however, is not supported by the data reported by Froesch et al.[2]. In an attempt to solve this problem we have measured the K_m values and maximal activities for Fru-1,6-P_2 in liver biopsies of children suffering from HFI.

Van den Berghe et al.[3] have reported the inhibition of phosphorylase *a* by Fru-1-P. This was tested with purified phosphorylase *a* from dog liver. We have used a 10,000 x g supernatant of a sonicated homogenate of human leucocytes to investigate the inhibition of Fru-1-P on the phosphorylase reaction and the dependency of the velocity on the concentration of P_i. The use of leucocytes is justified because in previous studies[4,5] it was found that human leucocyte phosphorylase resembles human liver phosphorylase.

MATERIALS AND METHODS. Liver biopsies from the children with HFI were obtained by needle biopsy (case A.v.L.), open surgery (case W.v.E.) and immediately after death (case B.B.). The control liver samples were obtained by needle biopsy or by surgery for other reasons. The samples were homogenized in 0.25 M sucrose and 10 mM Tris pH 7.4 and the total homogenate was used for the enzymatic analyses.

Leucocytes were isolated from heparinized blood according to the method of Wyss et al.[6]. The final preparation was homogenized in 0.05 M NaF, sonicated for 1 min and centrifuged for 10 min at 10,000 g. The supernatant was used for the enzymatic analyses.

The aldolase activities with the two substrates were measured by following the decrease in absorbancy at 340 nm of NADH in the complete assay with triosephosphate isomerase and glycerolphosphate dehydrogenase[7]. The activity of phosphorylase was measured according ref. 9. Protein determination was performed according to the method of Lowry et al.[8].

RESULTS

Fig. 1 shows the saturation plots of aldolase B from normal and HFI livers. It is clear that two patients (W.v.E. and B.B.) have a very low activity compared to the controls. At higher Fru-1-P concentrations these cases exhibit some activity. From this figure it can be concluded that the aldolase B in HFI livers has a markedly increased K_m value. For this reason the enzyme exhibits practically no activity. The saturation plot of the third case (A.v.L.) also shows that aldolase has an increased K_m value for Fru-1-P, but not to that extent as for the other two cases. Yet, this patient showed the clinical features characteristic for HFI. A Fru-1,6-P_2ase deficiency was excluded by means of loading experiments with L-alanine and glycerol.

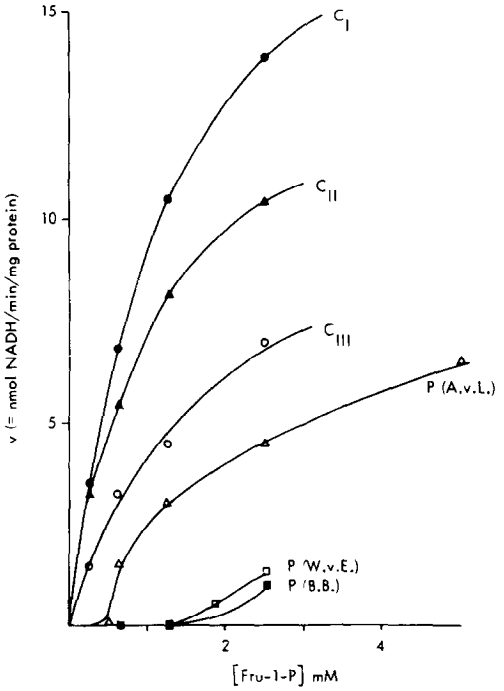

Fig. 1. The saturation curve for human liver aldolase from controls and HFI livers. C are controls. P are patients.

TABLE I shows the kinetic parameters of liver aldolase with Fru-1,6-P_2 as the substrate from control and HFI livers. The K_m value for controls is between 4.5 to 11.6 μM, in contrast to the K_m values for the HFI livers that are markedly increased, at least in these three cases. Also the V_{max} values are given. Two cases showed normal V_{max} values, in contrast to the findings of Hers and Joassin[1], but in agreement with those of Froesch et al.[2]. Only the third case (A.v.L) showed a V_{max} value of about 20% of the controls.

TABLE I

THE KINETIC PARAMETERS OF Fru-1,6-P_2 ALDOLASE IN NORMAL AND HFI PATIENTS.

	V_{max} (nmoles/min/ mg protein)	K_m (uM)
C_I	27	4.5
C_{II}	13	8.5
C_{III}	23	11.6
P(W.v.E.)	20	150
P(B.B.)	18	100
P(A.v.L.)	5	50

After administering fructose to HFI children the blood glucose level drops very quickly. This cannot be explained by a blocked gluconeogenesis but it is more likely due to an impaired glycogenolysis. From other experiments an inhibition of phosphoglucomutase could be excluded[11]. As was mentioned in the introduction, leucocytes were taken to investigate the effect of Fru-1-P, at various P_i concentrations, on phosphorylase. Fig. 2 shows the effect of Fru-1-P on the phosphorylase reaction at various P_i concentrations. It shows that the inhibition is non-competitive with a K_i value of about 6 mM. For the purified phosphorylase of dog liver a competitive inhibition was reported[3,12]. From Fig. 2 it can be concluded that

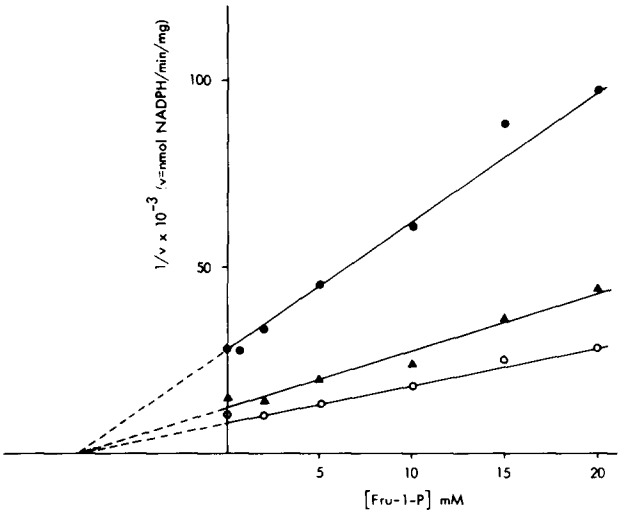

Fig. 2. 1/v vs [Fru-1-P] plot of human leucocytes phosphorylase at various P_i concentrations. ●-●, $[P_i]$ = 2 mM; ▲-▲, $[P_i]$ = 4 mM and ○-○, $[P_i]$ = 10 mM.

when the concentration of Fru-1-P reaches the value of 10 mM and the P_i concentration drops from 10 to 2 mM, a physiological concentration, the phosphorylase activity is inhibited for 83%.

Recently Lemonnier et al.[13] reported that extraction of aldolase from HFI liver with mercaptoethanol normalizes the affinity for Fru-1-P. From these experiments they concluded that thiol groups are involved. However, we were not able to confirm this conclusion. It should be mentioned, however, that in our cases the livers had been stored frozen for about 2 years when the mercaptoethanol extraction studies were carried out. Furthermore no influence of oxidized glutathione has been found for normal liver aldolase both with Fru-1-P and Fru-1,6-P_2 as substrate.

DISCUSSION

From the data shown it can be concluded that the Fru-1-P aldolase from livers of children with HFI has a markedly decreased affinity for the substrate Fru-1-P except one case (A.v.L.) who only shows a slightly decreased enzyme activity, while having clinically the typical HFI symptoms.

For aldolase activity with Fru-1,6-P_2 as substrate Hers et al.[1] found a decreased activity, whereas Froesch et al.[2] found normal

enzyme activity in HFI patients as compared to controls. As for our results, the data of the patient (A.v.L.) mentioned above are in agreement with the findings of Hers, the data of the other two patients with those of Froesch. One might speculate that the HFI-syndrome consists of two different "subtypes" of enzyme deficiency. The fact that the K_m for Fru-1,6-P_2 is also markedly increased is rather surprising. This K_m increment has no apparent effect on the glycolytic flux which remains undisturbed as long as the patient is deprived of fructose. The hypoglycemia induced by fructose loading can be explained by a drop in the inorganic phosphate concentration and an increase in the Fru-1-P concentration. Van den Berghe et al.[3] showed that the phosphorylase reaction can be inhibited by 70%. For the phosphorylase from human leucocytes, which resembles the human liver phosphorylase, we found an inhibition of about 80%. The inhibition by Fru-1-P is of a non-competitive nature, which is in contrast to the competitive inhibition found for dog and mouse liver[3,12,14].

ACKNOWLEDGEMENT

The authors are indebted to Prof. Dr. W.C. Hülsmann for support and advice. Miss A.C. Hanson is thanked for the preparation of the manuscript.

REFERENCES

1. Hers, H.G. and Joassin, G. (1961) Enzymol. Biol. Clin. 1, 4-14.
2. Froesch, E.R., Wolf, H.P. and Baitsch, H. (1963) Am. J. Med. 34, 151-167.
3. Van den Berghe, G., Hue, L. and Hers, H.G. (1973) Biochem. J. 134, 637-645.
4. Fernandes, J. and Pikaar, N.A. (1972) Arch. Dis. Childhood 47, 41
5. Koster, J.F., Fernandes, J., Slee, R.G., Van Berkel, Th.J.C. and Hülsmann, W.C. (1973) Biochem. Biophys. Res. Commun. 53, 282-290.
6. Wyss, S.R., Koster, J.F. and Hülsmann, W.C. (1971) Clin. Chim. Acta 35, 277-280.
7. Gracy, R.W., Lacko, A.G., Brox, L.W., Adelman, R.C. and Horecker, B.L. (1970) Arch. Biochem. Biophys. 136, 480
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.C. (1951) J. Biol. Chem. 193, 265
9. Hülsmann, W.C., Oei, T.L. and Van Creveld, S. (1961) Lancet 2, 581

10. Huijing, F. (1964) Clin. Chim. Acta 9, 269
11. Cornblath, M. Rosenthal, I.M., Reisner, S.H., Wybregt, S.H. and Crane, R.K. (1963) New Engl. J. Med. 269, 1271
12. Kaufmann, U. and Froesch, E.R. (1973) Eur. J. Clin. Invest. 3, 407-413.
13. Lemonnier, F., Gregori, C. and Schapira, F. (1974) Biochem. Biophys. Res. Commun. 61, 306.
14. Thurston, J.H., Jones, E.M. and Hauhart, R.E. (1974) Diabetes 23, 597-604.