POSSIBLE ROLE OF THIOL GROUPS IN THE ABNORMAL KINETICS OF ALDOLASE IN HEREDITARY FRUCTOSE INTOLERANCE

F. Lemonnier, C. Gregori and F. Schapira
With the technical collaboration of M.F. Szajnert

Institut de Pathologie Moléculaire, 24 rue du Fbg St Jacques PARIS 75014 - France.

Laboratoire associé au Centre National de la Recherche Scientifique, Groupe U.129 de l'Institut National de la Santé et de la Recherche Médicale.

Received September 9,1974

SUMMARY : In Hereditary Fructose Intolerance, the apparent Km of liver aldolase for Fructose-1-Phosphate was shown to be very high. The addition of β mercaptoethanol normalizes this low affinity. It seems that the primary defect of this genetic disease involves directly or indirectly the thiol groups of aldolase B.

Hereditary Fructose Intolerance (HFI) is an inherited metabolic disease due to a deficiency of aldolase "B" (liver-type aldolase, EC 4.1.2.7) (2) (4). The activity towards Fructose 1-6-diphosphate (Fru-1-6-P_2) falls to about 20% of the normal value ; principally, the activity towards a more specific substrate Fructose 1-Phosphate (Fru-1-P) is only about 3% of the normal range. Characteristically, the aldolase activity ratio Fru 1-6-P_2/Fru-1-P, which is normally very constant, between 1.0 and 1.3,is raised, and varies between 2.5 and 15.

Our group has demonstrated that the elevation of this ratio is due to the presence, in relatively high amounts, of the two other types of aldolase : aldolase A (Muscle-type : EC 4.1.2.13) and aldolase C (Brain type), both aldolases being more active on Fru 1-6 P_2 than on Fru 1 P. These aldolases are normally abundant in fetal liver, and probably persist without change in livers with HFI (10) (12).

We have shown, in livers of children with HFI, the presence of a protein immunologically related to aldolase B (8) (11), but

almost deprived of activity. Moreover, we were able to estimate the apparent Michaëlis constant (Km) for the most specific substrate, Fru-1-P, of the residual enzyme : in all cases tested we have found that the apparent Km was abnormal : between 15 and 30 mM instead of about 4 mM for normal liver. This kinetic abnormality was not due to Aldolase C (whose activity towards Fru 1 P is not negligible) because the affinity for this substrate of aldolase from livers with HFI remains low after the action of specific antiserum antialdolase C. We have also verified that it was not due to the environment.

This report shows the effect of β Mercaptoethanol on the kinetics of altered aldolase B.

MATERIAL AND METHODS

Samples of livers from children with clinical and biological signs of HFI were obtained by needle biopsy and immediately frozen (1). Samples of normal livers were removed during surgery for other diseases. Human aldolase B was prepared from livers removed "post mortem" ; the purification of the enzyme was performed according to a modification of the methods described for the purification of rabbit aldolase B by Morse and Horecker (7) and by Penhoët et al (9). Liver biopsies were extracted with 50 volumes of cold water, with or without 10 mM β Mercaptoethanol.

Aldolase activities towards the two substrates were estimated by, either a modification of the colorimetric method of Sibley and

(1) We thank Drs. Alagille, Laigle, Odievre, Polonovski and Verger for allowing us to study biopsies from their patients and for communicating their clinical studies.

Lehninger (13) or by following the decrease in absorbance at 340nm of NADH in a coupled reaction with α glycero phosphate dehydrogenase by the method of Blostein and Rutter (1). Apparent and true

Michaëlis constants were estimated from the Lineweaver and Burk curves with Fru-1-P as substrate.

RESULTS

Table 1 gives the mean value of F1P aldolase activity and the

TABLE I

KINETIC PROPERTIES OF NORMAL HUMAN ALDOLASE B, BRAIN ALDOLASE AND ALDOLASE OF HFI LIVERS

	ACTIVITY in I.U. (per mg of pure protein or per g of fresh tissue)		APPARENT Km/F1P (mM)	
	without β Mercaptoethanol	with β Mercaptoethanol	without β Mercaptoethanol	with β Mercaptoethanol
Purified Human Aldolase B	0.45	0.45	5.0	5.0
Normal liver extracts (6 different biopsies)	6 to 10	6 to 12	3.0 to 5.0	3.0 to 5.0
Brain extracts (4 brains : surgery or post-mortem)	0.25 to 0.4	0.25 to 0.4	14 to 18	14 to 18
Livers with HFI extracts Cases : P.A.	0.I5	0.22	>20	<3
X.M.	0.09	0.50	15 to 20	<3
P.J.	0.I8	0.26	15 to 20	<3
S.C.	avout 0.03	0.08	>20	2 to 4
S.M.*	0.20	0.36	15 to 20	2 to 4
C.M.*	0.36	0.22	>20	< 3

* Measurements with and without β Mercaptoethanol were performed in both cases on biopsies made with a one year gap.

apparent Km for Fru 1 P of aldolase extracted from "normal" human livers ; of purified human aldolase B and of aldolase C extracted from human brain with and without β Mercaptoethanol. Table 1 gives also the activity and apparent Km's towards Fru-1-P (estimated by the same methods) of aldolases in 6 different livers with HFI.

Activities towards Fru-1-P of pure aldolase B and of brain aldolase were not increased by the addition of β Mercaptoethanol 10 mM. We have inconstantly found a slight increase of Fru 1 P aldolase activity by adding β Mercaptoethanol to normal liver extracts. This increase was constant (although variable in intensity), for aldolase from livers with HFI*.

The apparent Km for Fru-1-P of aldolase in normal liver extracts was between 3 and 5mM. The Km of purified aldolase B was found to be about 5mM. Extraction and incubation with β Mercaptoethanol did not change these values.

The apparent Km for Fru-1-P of brain aldolase is higher : about 15 mM. It must be pointed out that like the Km of normal aldolase B, it does not change with the addition of β Mercaptoethanol.

Principally Table 1 shows that, for all aldolases from livers with HFI which we were able to study, the apparent Km without β Mercaptoethanol was high : between 15 and 20 mM. When the altered enzyme was extracted with β Mercaptoethanol 10 mM, the affinity seemed to be normalized : the apparent Km was lowered to about 3mM, or even less.

DISCUSSION

In the present report, we confirm our previous results on the abnormal affinity for Fru 1 P of aldolase from livers with

*Except in one case where extractions with and without β Mercaptoethanol were performed on biopsies made with a one year gap.

HFI. Although the determination was obviously not very precise (due to the very small amount of liver obtained from needle biopsy and the very low Fru-1P aldolase activity), it is certain that the affinity was lowered : the Fru 1 P concentration giving one half of the maximum observed rate was about 20 mM when aldolase was extracted without β Mercaptoethanol ; it was of about 3mM with this reagent.

Our previous results on the persistent abnormal kinetics of aldolase from livers with HFI after the action of an antiserum anti aldolase C are strengthened by our present findings. The difference between the action of β Mercaptoethanol on brain aldolase and on aldolase from livers with HFI, is supplementary evidence that the abnormal kinetics for F1P of aldolase in livers with HFI is actually due to an altered aldolase B and not to aldolase C present in these livers.

This alteration seems to be related to the thiol groups, since it is corrected by the addition of SH reagents.

In order to understand this effect, we have compared our results with the previous studies on cysteins in rabbit aldolases A and B.

Rabbit aldolase A contains 32 cysteins per mole or 8 per subunit (6). The loss of activity by blocking -SH groups would result principally from structural changes (15) (16) (19) ; thiol groups would act to maintain protein conformation. Steinman and Richards (14) have shown that the sulfhydryl groups could be subdivided into three classes : "exposed", "protected" by substrates and "buried". They have also found that Km for Fru 1-6 P_2 and for Fru 1 P of aldolase A increases significantly when the two "protected" -SH groups are modified by disulfide monosulfoxide reagents. The thiol groups of rabbit aldolase B were not so extensively studied ; ne-

vertheless, the structure at the active centers of both aldolases A and B, is known to be very similar (5) (7). On the other hand, the structure of the active centers is similar in several species (17).

Gurtler et al have purified human aldolase B and have studied its amino acid composition : it was very similar to that of rabbit aldolase B and even of rabbit aldolase A (3).

Consequently it is possible to compare our results on the action of -SH reagents on the affinity of aldolase in HFI, with the results of Steinman and Richards with rabbit aldolase A. It seems that in HFI there is a modification of the -SH groups.

The reports on the influence of -SH reagents on the affinity of a mutant enzyme in eucaryotes are very scarce.

Recently, Van Berkel et al (18) have shown that incubation with 10 mM β Mercaptoethanol of erythrocyte pyruvate kinase, from a patient with hemolytic anemia, converted the abnormal kinetics of this deficient enzyme towards phosphoenol pyruvate into a normal kinetics.

Our results, together with the previous ones showing the presence in livers with HFI of a protein immunologically related to aldolase B, but with a very low enzymic activity, give evidence for a structural mutation of this enzyme in HFI. It seems that the primary defect implicates, directly or indirectly, some thiol groups with, as a consequence, a decreased affinity for the specific substrate Fru 1 P, this defect being corrected by reducing compounds.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (ATP n° 8).

REFERENCES

1. Blostein, R., and Rutter, W.J. (1963) J. Biol. Chem. 238, 3280-3285.
2. Froesch, E.R. (1972) The Metabolic Basis of Inherited Diseases, pp. 131-148, Stanbury J.B., Wyngaarden, J.B. and Fredrickson, D.S. ed. New-York.
3. Gurtler, B., Bally, C., and Leuthardt, F. (1971) Z. Hoppe Seyler's Physiol. Chem. 352, 1455-1462.
4. Hers, H., and Joassin, G. (1961) Enzymol. Biol. Clin. 1, 4
5. Horecker, B.L. (1970) in Sols, A. and Grisolia S. Metabolic regulation and enzyme action. Acad. Press, 19, 181-189.
6. Horecker, B.L., Tsolas, O., and Lai, C.Y. (1972) The enzymes pp. 213-218, Boyer ed. 7.
7. Morse, D.E., and Horecker, B.L. (1968) Arch. Biochem. Biophys. 125, 942-962.
8. Nordmann, Y., Schapira, F., and Dreyfus, J.C.D. (1968) Biochem. Biophys. Res. Commun. 31, 884-889.
9. Penhoet, E.E., Kochman, M., and Rutter, W.J. (1969) Biochemistry 8, 4391-4395.
10. Schapira, F., and Dreyfus, J.C.D. (1967) Rev.Fr.Et.Clin. Biol. 12, 486-489.
11. Schapira, F., Hatzfeld, A., and Gregori, C. (1974) Enzyme 18, 73-83.
12. Schapira, F., Nordmann, Y., and Gregori, C. (1972) Acta Med. Scand. Suppl. 542, 77-83.
13. Sibley, J.A., and Lehninger, A.O. (1949) 177, 859-872.
14. Steinman, H.M., and Richards, F.M. (1970) Biochemistry 9, 4360-4372.
15. Swenson, A.O., and Boyer, P.O. (1957) J. Am. Chem. Soc. 79, 2174-2179.
16. Szabolcsi, G., and Biszku, E. (1961) Biochim. Biophys. Acta 48, 335-341.
17. Ting, S.M., Lai, C.Y., and Horecker, B.L. (1971) Arch. Biophys. Biochem. 144, 476-484.
18. Van Berkel, J.C., Staal, G.E.J., Koster, J.F., and Nyessen J.G. (1974) Biochim. Biophys. Acta 334, 361-367.
19. Zavodsky, P., Biszku, E., Abaturov, V., and Szabolcsi, G. (1972) Acta Biochim. Biophys. Acad. Sci. Hung. 7, 1-10.