SUBSTRATE SPECIFICITY OF TPN L-HEXONATE DEHYDROGENASE

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In the course of comparative studies on the substrate specificity of TPN L-gulonic dehydrogenase (Mano et al., 1959) which have been made in order to differentiate the enzyme from other enzymes similar in action (e.g. Hers, 1960), it has been found that the enzyme shows a specificity which might be termed "TPN L-hexonate dehydrogenase." This enzyme, through its broad specificity, may play an important role in carbohydrate metabolism.

The enzyme preparation used in the present experiment was purified from rat liver as described in the previous communication and the reactions were carried out under the same conditions as reported before (Mano, et al., 1959). Although the preparation was highly purified, it was proved to be broadly specific. As reported previously, TPN or TPNH could not be replaced by DPN or DPNH in the reaction.

Specificity in the reduction process : TPN L-hexonate dehydrogenase acts on alduronic acids of which the carbon number varies from six to three; D-glucuronate, D-galacturonate, D-mannuronate, L-iduronate, L-threuronate, and DL-tartronic semialdehyde, with the exception of five, D-xyliuronate, D-lyxuronate, and L-arabiuronate. It does not act on the corresponding keturonates, such as D-fructuronate, D-tagaturonate, 2-keto-L-gulonate, and its methyl ester, 2-keto- and 5-keto-D-gluconate, keto-DL-erythronate*, and hydroxypyruvate, and other keto acids as α-ketobutyrate, pyruvate and acetoacetate. The enzyme

* The compound corresponds to "Oxyerythronsäure" named by Neuberg (1910).

also catalyzes the reduction of such derivatives as lactones, esters, and an amide of the above mentioned alduronic acids, D-glucurono-γ-lactone, ethyl D-glucuronate, D-glucuronamide, methyl D-galacturonate, D-mannurono-γ-lactone, L-idurono-γ-lactone, and methyl diacetyl L-threuronate. The enzyme was proved to be also active on glyoxylate, malonic semialdehyde, succinic semialdehyde, and glutaric semialdehyde. Various osones derived from hexose, D-glucosone and D-galactosone, are accessible, whereas those from pentose, D-xylosone and L-arabinosone, are not. Methylglyoxal has been proved to be the most active among the compounds tested. Of various aldomonosaccharides examined, those with carbon number of three and four, D- and L-glyceraldehyde, D-erythrose, D-threose, and DL-glyceraldehyde-3-phosphate, are reactive, while those with carbon number from five to six, D- and L-xylose, D- and L-arabinose, D- and L-lyxose, D-glucose, D- and L-galactose, D-mannose, L-rhamnose, L-fucose, D-gluco- and D-galactosamine, and D-glucose- and D-galactose-6-phosphate, are not, with the exception of D-ribose which showed a slight activity. Glycolaldehyde is also reactive. Various ketomonosaccharides, sedoheptulose, L-sorbose, D-fructose, D-tagatose, and dihydroxyacetone, do not serve as substrate. The enzyme is of considerable activity on dialdehydes, glyoxal, malonic dialdehyde, succinic dialdehyde, and glutaric dialdehyde. D-xylo-dialdopentofuranose is also accessible. Typical aldehydes, such as acet-, propion-, n-butyr- and n-valeraldehyde, are somewhat reactive; those activities are almost in parallel with the chain length. This pattern of specificity has been demonstrated to be irrespective of configuration of these compounds. These observations suggest that TPN L-hexonate dehydrogenase attacks the carbohydrate of oxo-form and other aldehyde compounds, except those derived from pentose in both cases, in other words, free aldehyde group is essential for the activity. The activity for uronate may be considered to be of special case. That the oxo-form is apparently specific for the enzyme is further confirmed by experiments in which the effectiveness of 2,3,4,5,6-pentaacetyl D-glucose and ineffectiveness of 2,3,5,6-tetraacetyl D-glucose have been clearly demonstrated. Furthernore, the fact that the activities with some sugars, D-ribose, L-arabinose, D-gala-

ctose, and D-glucose at higher concentrations, 3.3×10^{-2} M, have been in good proportion to the contents of oxo-form shows clearly this relation. Although it has recently been reported that D-glucurono-γ-lactone in aqueous solution exists nearly completely as oxo-form (Kawada, unpublished), data on other related compounds are not available at present. The reaction products, i.e. L-gulonate from D-glucuronate, L-gulono-γ-lactone from each D-glucurono-γ-lactone and ethyl D-glucuronate, L-galactono-γ-lactone from methyl D-galacturonate, and D-xylose from D-xylo-dialdopentofuranose, have been identified by means of paper chromatography with four solvent systems. The formation of L-gulono-γ-lactone from D-glucurono-γ-lactone and ethyl D-glucuronate has also been comfirmed by coupling reaction with L-gulonolactone dehydrogenase leading to L-ascorbate. The possibility that D-fructose is formed from D-glucosone and acetol from methylglyoxal has been suggested through the use of the reactions of Roe et al. (1949) and of Baudisch (1918), respectively.

Specificity in the dehydrogenation process : The enzyme acts exclusively on the L-form of both hexonic acids and their lactones, L-gluconate and its δ-lactone, L-galactonate, L-mannonate, L-gulonate, and L-idonate and their γ-lactones, with the exception of minute activity of D-mannonate and its γ-lactone. In general, the activity is higher in acid form than in lactone form. Another exception, ineffectiveness of L-rhamnonate and its γ-lactone as substrate, would be indicative of necessity of the presence of alcohol group at C-6 for the enzyme activity. Compounds of aldonate and their derivatives other than those described above are all ineffective: D-gluconate and its δ- and γ-lactone; D-α- and β-glucoheptonate, D-allonate, D-altronate, D-gulonate, D-idonate, D-galactonate, D-talonate, D-ribonate, D- and L-arabonate, D- and L-xylonate, D- and L-lyxonate, L-threonate, and their γ-lactones; DL-erythronate, DL-glycerate, glycolate, N-acetyl neuraminate, β-hydroxypropionate, and β-hydroxybutyrate. Various polyalcohols, such as D-sorbitol, D-mannitol, meso-dulcitol, meso-adonitol, meso-erythritol, L-threitol, glycerol, and ethylene glycol, are also inactive. Of the probable products in the reduction process from effective substrates, the various ketoses des-

cribed above are not oxidized. This pattern of specificity does not change at the optimum pH, 9.6. All the facts indicate that for the dehydrogenation of hexonate the levo configuration of the hydroxyl group at C-5 would be essential.

As far as were tested in animal tissues, these pattern of specificity is irrelevant in principle to the source of the enzyme. Thus only the reactions such that the reduction product is L-hexonate are reversible while others are irreversible. The reason why alduronates and osones from pentose are not accessible remains to be elucidated. Detailed quantitative data and a discussion on the discrimination and the role of the enzyme in carbohydrate metabolism will be presented elsewhere (Mano et al., unpublished).

Addendum : Data presented in the previous communication (Mano et al., 1959) on dehydrogenation must be corrected as in this paper.

References

Baudisch, O., Biochem. Z., 89, 279 (1918)

Hers, H. G., Biochim. et Biophys. Acta, 37, 120 (1960)

Kawada, M., Personal communication

Mano, Y., Yamada, K., Suzuki, K., and Shimazono, N., Biochim. et Biophys. Acta, 34, 563 (1959)

Mano, Y., Suzuki, K., and Yamada, K., J. Biochem., 47, in press

Neuberg, C., Biochem. Z., 24, 166 (1910)

Roe, J. H., Epstein, J. H., and Goldstein, N. P., J. Biol. Chem., 178, 839 (1949)