Substrate specificity of DPN-L-gulonic acid dehydrogenase

The enzymic conversion of L-gulonic acid to L-xylulose in mammalian tissues has been investigated by several workers[1-3]. ISHIKAWA in our laboratory first purified the enzyme, DPN-L-gulonic acid dehydrogenase, from guinea-pig liver and studied its properties[2]. ASHWELL *et al.* also reported the purification and properties of the enzyme from hog kidney[3]. The substrate specificity of the enzyme, however, has not yet been investigated satisfactorily by these authors. In the course of recent studies in our laboratory concerning a series of aldonic acids, elucidation of the substrate specificity of the enzymes participating in the metabolism of the aldonic acids was required. The present paper deals with this problem in respect to DPN-L-gulonic acid dehydrogenase.

The enzyme was purified from guinea-pig liver exactly according to the purification procedures already described by ISHIKAWA[2]. The assay method was also the same as his description. Mainly aldonic acids: trionic, tetronic, pentonic, hexonic and heptonic acids, and some related compounds were examined as substrates. The results are shown in Table I. Of these compounds tested L-gulonate, D-gluconate, D-mannonate, L-idonate, D-xylonate, D-lyxonate, DL-erythronate, L-threonate and DL-β-hydroxybutyrate were effective. In all these compounds the hydroxyl group of the C-3 has a L-configuration and the hydrogen of the C-4 occupies a L-position. Those in which both hydroxyl groups of the C-3 and C-4 have L-configurations were not effective. Lactones corresponding to the above compounds were also effective, although to a much less extent, but considering that under the conditions of our assay method spontaneous degradation of lactones to the corresponding acids was inevitable and that some time lag was observed in the initial stage of the reaction, the effectiveness of lactones might be regarded as secondary. Also the following might be suggested: 2-keto, as well as 5-keto aldonic acids and the compound in which the terminal carbon is aldehyde do not serve as a substrate. In the course of our present study SMILEY *et al.* reported on the same problem, *i.e.* the substrate specificity of the enzyme and the reverse reaction catalyzed by the enzyme[4]. They concluded that the enzyme is active upon all hexonic, pentonic and tetronic acids examined in which the hydroxyl group of the C-3 has a L-configuration, and that the reverse reactions from 2,3-diketo-L-gulonate, 2,3-diketo-D-gluconate, 2,3-diketo-D-glucoheptonate and acetoacetate to the corresponding C-3 hydroxy acids occur. In our study, however, the enzyme is not active upon the compounds such as D-galactonate, D-talonate and L-arabonate in which the hydroxyl group of the C-3 has a L-configuration. Therefore, we are of opinion that the simple condition of the C-3 L-hydroxyl group is not sufficient, but the configuration of C-3 L-hydroxy and C-4 L-hydrogen are necessary for the substrate. We also tried to examine spectrophotometrically the reverse reaction catalyzed by the enzyme using 2,3-diketo-L-gulonate, 2,3-diketo-D-gluconate and acetoacetate as a substrate. However, under various conditions examined the reverse reaction was not found. The difference between the observations of SMILEY *et al.* and ours might be attributed to the difference of the source of the enzyme.

D-Gluconate was previously reported ineffective, but it has now been found that

Abbreviations: DPN, diphosphopyridine nucleotide; Tris, tris(hydroxymethyl)aminomethane.

TABLE I

SUBSTRATE SPECIFICITY OF DPN-L-GULONIC ACID DEHYDROGENASE

The reaction mixture contained Tris buffer, 100 μmoles; $MnCl_2$, 0.2 μmole; DPN, 0.65 μmole; substrate, 10 μmoles, or 20 μmoles when the substrate was DL-form; enzyme, 0.1 ml (approx. 100 units*). Total vol., 3.0 ml, pH 8.4. Incubation for 5 min at 25°. The change in absorbancy at 340 mμ due to the reduction of DPN was determined.

Substrate	*Relative activity of that of* L-*gulonate as 100*	*Substrate*	*Relative activity of that of* L-*gulonate as 100*
DL-Glycerate	0		
DL-Erythronate	17.1		
		L-Threonate	7.8
DL-β-Hydroxybutyrate	14.4		
D-Ribonate	0		
D-Arabonate	0	L-Arabonate	0
D-Xylonate	15.5	L-Xylonate	0
D-Lyxonate	44.6	L-Lyxonate	0
D-Allonate	0		
D-Altronate	0		
D-Gluconate	19.0	L-Gluconate	0
2-Keto-D-gluconate	0		
5-Keto-D-gluconate	0		
D-Glucuronate	0		
D-Mannonate	5.1	L-Mannonate	0
D-Gulonate	0	L-Gulonate	100
		2-Keto-L-gulonate	0
		Methyl 2-keto-L-gulonate	0
D-Idonate	0	L-Idonate	68.8
D-Galactonate	0	L-Galactonate	0
D-Talonate	0		
D-α-Glucoheptonate	0		
D-β-Glucoheptonate	0		

* Unit is as defined by ISHIKAWA[2].

this compound was 20 % as active as L-gulonate. The reaction product from D-gluconate is probably D-ribulose. This reaction is very interesting in connection with the metabolism of non-phosphorylated sugars. The identification of the reaction product is now under investigation in our laboratory.

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