The enzymic conversion of D-glucuronate to L-ascorbate and L-xylulose in animal tissues*

This report characterizes the enzymic reaction pattern in the conversion of D-glucuronate to L-ascorbate[1]- and L-xylulose[2] which occurs in liver and kidney.

Partially purified preparations from hog kidney catalyze the reversible reduction of D-glucuronate to L-gulonate, which is specifically linked to TPN:

$$\text{TPNH} + \text{D-glucuronate} + \text{H}^+ \rightleftharpoons \text{TPN}^+ + \text{L-gulonate} \quad \text{(i)}$$

The free acid anions are the immediate substrates and the γ-lactones appear to be attacked only after hydrolysis. D-galacturonate is reduced and L-galactonate is oxidized at rates of the same order as for D-glucuronate and L-gulonate.

The conversion of L-gulonate to L-ascorbate appears to occur in two separate stages:

$$\text{L-gulonate} + \text{DPN}^+ \rightleftharpoons [\text{3-keto-L-gulonate}] + \text{DPNH} + \text{H}^+ \quad \text{(ii)}$$

$$[\text{3-keto-L-gulonate}] \longrightarrow \text{L-ascorbate} + \text{H}_2\text{O} \quad \text{(iii)}$$

However, the postulated intermediate, 3-keto-L-gulonate, may alternatively undergo decarboxylation to L-xyluloxe and CO_2[2].

$$[\text{3-keto-L-gulonate}] \rightleftharpoons \text{L-xylulose} + \text{CO}_2 \quad \text{(iv)}$$

These formulations are supported by the following evidence: When L-gulonate and DPN were incubated with relatively high concentrations of rat liver or pig kidney fractions, then DPNH, CO_2, and a pentose, identified chromatographically as xylulose[3], were formed according to reactions (ii) + (iv) (Table I). Under these conditions, no L-ascorbate was formed. This reaction is specific for DPN and for the L-stereoisomer of gulonate and galactonate; the lactones were active only after hydrolysis. When $^{14}CO_2$ was incubated with L-gulonate, DPN, and the enzyme fraction, a DPN-dependent incorporation of ^{14}C into the carboxyl carbon of L-gulonate was observed. These enzyme preparations do not contain the xylitol dehydrogenases described by HOLLMAN AND TOUSTER[4]. These facts indicate that the xylulose formed is the L-stereoisomer. These reactions can therefore account for the formation of L-xylulose from D-galacturonate[5] and L-gulonate[6,7].

TABLE I

FORMATION OF ASCORBATE AND XYLULOSE FROM L-GULONATE

Enzyme	*DPNH*	*L-xylulose*	*CO_2*	*H+*	*Ascorbate*
1. Rat liver (16.8 mg)	207	167	208	208	0
2. Pig kidney (13.5 mg)	183	159	—	—	2
Pig kidney (0.54 mg)	2340	1330	—	—	432

The system contained 15 μmoles $KHCO_3$, 50 μmoles potassium L-gulonate, 20μmoles DPN, and enzyme as noted in a volume of 1.3 ml. The gas phase was 95% N_2-5% CO_2, and the reaction period was 1 h at 37°. Xylulose was estimated by the method of KULKA[8] and ascorbic acid by the method of ROE AND KUETHER[12]. Data in mμmoles/mg enzyme.

On the other hand, when the concentration of the pig-kidney fraction in the test system was lowered (Table I), relatively less xylulose was formed from L-gulonate, but some of the latter was now converted into L-ascorbate, which was identified chromatographically. This finding suggests that the immediate oxidation product of L-gulonate has two pathways, the proportion depending on experimental conditions. Dilution of the enzyme presumably causes dissociation of a co-factor and/or inhibitor for one of the pathways.

The enzymic mechanism of ascorbic acid formation in animal tissues indicated by these experiments is in accordance with results of isotopic experiments[9] *in vivo*, but differs in some ways from that in plants[10]. The participation of these two reactions in metabolism of L-gulonate by primates and the guinea pig, which require dietary ascorbic acid, is being investigated[11].

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ADP-polynucleotide phosphorylase

The enzyme polynucleotide phosphorylase catalyzes the reversible polymerization of various nucleoside diphosphates to form synthetic polynucleotides containing one or more bases[1]. Both the enzyme and the polymers have been studied by a number of investigators[2–6]. Evidence presented here indicates that several enzymes may be involved in this overall reaction.

In Table I the results of experiments are presented in which each diphosphate was incubated with either a crude or partially purifed enzyme preparation. In the former cases there was high polymerizing activity toward ADP and CDP but much less toward GDP and UDP. Inorganic phosphate production greatly exceeded polymer formation, indicating the presence of phosphatases.

TABLE I

Each incubation vessel contained the following in a final volume of 0.32–0.35 ml: nucleoside diphosphate, 1.0 μmole; Mg^{++}, 0.46 μmole; EDTA, 0.05μmole; glutathione, 1.84 μmoles; glycine buffer, pH 10.1, 92 μmoles; and enzyme solution, 0.20 ml. Incubations were carried out for 90 min at 37° and stopped by boiling. The values reported are averages of the number of experiments indicated in parentheses.

	Substrate	*Polymer formed (μmole) as determined by:*		
		Chromatogram origin [a]	*Polymer* [b]	P_i [c]
Crude preparations	ADP* (9)	0.33	0.31	0.53
	GDP (4)		0.01	0.09
	UDP (4)		0.04	0.13
	CDP (9)		0.26	0.54
Partially purified preparations	ADP* (4)	0.30	0.25	0.35
	GDP (2)		0.00	0.00
	UDP (2)		0.00	0.03
	CDP (4)		0.03	0.04
	CDP + poly-C [d] (1)		0.00	0.04
	CDP + RNA [e] (1)		0.00	0.07

[a] The radioactivity remaining at the origin after paper chromatography.
[b] Ultraviolet-absorbing material precipitated with $HClO_4$ and redissolved in buffer.
[c] Inorganic phosphate appearing.
[d] 0.10 μmole of poly-cytidylic acid was added to this vessel.
[e] 0.10 μmole of *M. lysodeikticus* nucleic acid was added to this vessel.