The enzymic conversion of L-gulonate to L-ascorbate by rat-liver enzymes

In earlier communications[1,2] it was reported that L-gulonate is oxidized by a DPN-linked L-gulonate dehydrogenase in liver and kidney preparations to L-xylulose and L-ascorbate. The L-ascorbate so found was identified by paper chromatography[3] and estimated by the ROE AND KUETHER method.[4] However, recently it has been found that radioactive "L-ascorbate" formed from L-[1-^{14}C]gulonate by the action of DPN-gulonate dehydrogenase is not identical with authentic L-ascorbate when chromatographed on Dowex 1 columns. Although the identity of the chromogen has not yet been established, this report characterizes the enzymic reaction pattern in the conversion of L-gulonate to *true* L-ascorbate in rat-liver extracts.

The data in Table I demonstrate that rat-liver microsomes, previously shown to be involved in the synthesis of L-ascorbate[1,5], contain an enzyme, probably similar to the autooxidizable flavoprotein found in plants[6], which in the presence of oxygen is able to oxidize L-gulono-γ-lactone to L-ascorbate. The radioactive L-ascorbate formed from L-[1-^{14}C]gulono-γ-lactone was identified with certainty by paper chromatography[3], chromatography on Dowex 1 [7,8], and recrystallization from ethanol-water to constant specific activity after addition of carrier L-ascorbic acid. This reaction was previously observed by BURNS *et al.*[5]; the present experiments (Table I) demonstrate that the reaction occurs with L-gulono-γ-lactone but not with free L-gulonate.

TABLE I

The system contained in a total vol. of 2.0 ml: 40 μmoles imidazole-HCl, pH 6.8; 1 μmole $MnCl_2$; 10 μmoles substrate, and where indicated: 3-times washed microsomes prepared by the method of SCHNEIDER AND HOGEBOOM[13] from 0.75 g rat liver, and/or 136 units aldonolactonase (1 unit = 1 μmole CO_2 released from a bicarbonate buffer/15 min with D-galactono-γ-lactone as substrate[9]). The aldonolactonase was prepared from rat-liver homogenates by isoelectric precipitation, heat treatment, and chromatography on cellulose CM columns. After a reaction period of 2 h at 37° ascorbate was determined by the method of ROE AND KUETHER[4].

Enzyme(s)	*Gas phase*	*Substrate*	*Ascorbic acid (μmoles)*
1. Microsomes	O_2	L-gulono-γ-lactone	5.70
Microsomes	N_2	L-gulono-γ-lactone	0.00
2. Microsomes	O_2	L-gulono-γ-lactone	6.10
Microsomes	O_2	L-gulonate	0.00
Microsomes and Aldonolactonase	O_2	L-gulonate	1.30
Aldonolactonase	O_2	L-gulonate	0.03
Microsomes and Aldonolactonase	O_2	L-gulono-γ-lactone	2.60

L-ascorbate is formed from free L-gulonate, however, if the microsomes are supplemented with a soluble heat-labile factor from the 105,000 $\times$ *g* supernatant fraction of rat liver (Table I). No other cofactors are required. On the other hand, this soluble factor inhibits the oxidation of L-gulono-γ-lactone to L-ascorbate by the microsomes (Table I). These facts suggested that the active factor in the soluble fraction is the aldonolactonase recently described[9], presumably capable of acting reversibly. Experimental support for such a role of the aldonolactonase was provided

Abbreviation: DPN, diphosphopyridine nucleotide.

by comparing the stimulation of formation of L-ascorbate from L-gulonate in the presence of microsomes with the aldonolactonase activity during a 60-fold purification of the factor; the activities were found to be parallel. The purified enzyme catalyzes the accumulation of L-gulonolactone from L-gulonate, measured by conversion to the hydroxamate following addition of alkaline hydroxylamine after the incubation period.

These results, which are consistent with isotopic experiments[10] and with the independent work of YAMADA *et al.*[11], indicate the following reaction sequence:

$$\text{L-gulonic acid} \underset{+\,H_2O}{\overset{-H_2O}{\rightleftharpoons}} \text{L-gulono-}\gamma\text{-lactone} \qquad \text{(i)}$$

$$\text{L-gulono-}\gamma\text{-lactone} \xrightarrow{O_2} \text{L-ascorbic acid} \qquad \text{(ii)}$$

These results thus demonstrate a role for the aldonolactonase present in liver and kidney of many species[9,11] and show that L-gulono-γ-lactone is the immediate precursor of L-ascorbate. On the other hand, L-gulonate rather than the lactone appears to be the direct precursor of L-xylulose[2,12].

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Transfer of radioactive sulfate from phosphoadenosine phosphosulfate to heparin

The description of a transplantable mouse mast-cell tumor by DUNN AND POTTER[1] suggested the possibility that this mastocytoma could be used for a study of heparin biosynthesis *in vitro*. Homogenates of this tumor were prepared, and incubated with inorganic $^{35}SO_4^=$. The heparin was extracted, and purified by paper chromatography, according to methods recently developed in this laboratory[2,3,4]. It was observed that, under certain conditions, labeled inorganic sulfate was incorporated into heparin by the tumor homogenate. These results were recently reported in part[3]. In view of the work by LIPMANN and co-workers on the role played by PAPS in a number of different

Abbreviations: PAPS, 3'-phosphoadenosine-5'-phosphosulfate; DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; UTP, uridine triphosphate.