Biodegradation of dehydro-L-ascorbic acid; 2,3-diketo-aldonic acid decarboxylase from rat liver

Decarboxylation of L-ascorbic acid at C-1 *in vivo*[1,2] and *in vitro*[1,3-5] has been shown to take place after its oxidation to DAA followed by its hydrolysis to DKG. The product of decarboxylation is reported as L-xylose[1] in liver or L-lyxonic acid[4] in kidney. The present communication deals with an enzyme, 2,3-diketo-aldonic acid decarboxylase, which was purified to confirm the reaction mechanism and the products of above decarboxylation. The decarboxylation activity was determined manometrically in 0.1 *M* phosphate buffer, pH 6.8, with 20 m*M* of DKG added from the side arm, at 37° under N_2. The enzyme activity was also estimated by the disappearance of DKG (0.2 m*M* in 0.01 *M* phosphate buffer) with 2,4-dinitrophenylhydrazine or with aniline citrate.

The supernatant fraction of rat-liver KCl homogenate was subjected to $(NH_4)_2SO_4$ fractionation (0.55–0.70 satn.), dialysis, and fractionation on a DEAE-cellulose column. The fraction eluted with 0.05 *M* NaCl in 5 m*M* phosphate buffer, pH 7.5, usually showed a specific activity 100 times that of the supernatant fraction (yield, 15 %). The activity was thermolabile but was resistant to dialysis, freezing, and treatment with SH inhibitors, alkali, charcoal and ion exchangers. Thiamine deficiency did not lower the enzyme activity. Metals were not needed in the reaction mixture though inhibition by chelating agents was recovered with Mg^{++} or Mn^{++}. The enzyme was found to be distributed in the kidney and liver of a rat, dog and hog, and was concentrated in the same $(NH_4)_2SO_4$ fraction.

Among the keto acids tested, substrates were limited to DKG or other 2,3-diketo-aldonic acids derived from ascorbic acid homologues. DAA was also decarboxylated but with a lag owing to its hydrolysis into DKG. The reaction products of DKG were absorbed with Dowex-1 column and eluted with formic acid. They were identified as lyxonic acid and probably xylonic acid on paper chromatograms with three solvent systems. Highly reductive spots were also found which were formed non-enzymically. The reaction was not reversed as shown by the use of $^{14}CO_2$.

The fact that two reaction products were obtained by the purified decarboxylation enzyme suggested at least three possibilities:

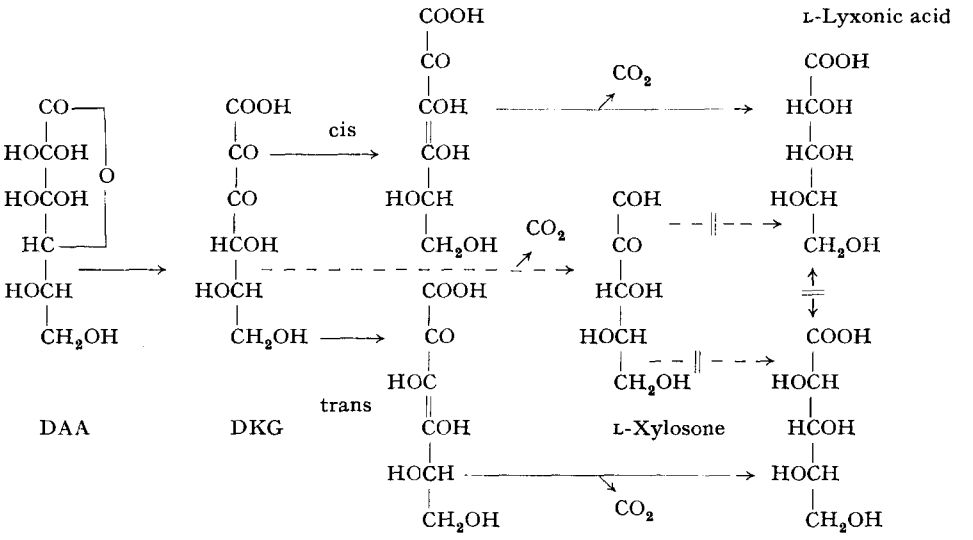
(1) Epimerization between L-lyxonate and L-xylonate. However, addition of one of these compounds did not produce the other.

(2) Intramolecular oxido-reduction of L-xylosone formed after enzymic decarboxylation. However, neither of the aldonates was formed from xylosone in this reaction mixture.

(3) Enolization of DKG before decarboxylation, which process results in the formation of two true substrates, *i.e. cis-* and *trans*-3,4-enediol-L-gulonic acid. The presence of the enediolform of DKG[6] in neutral solution was suggested by the pH-titration curve and by the reductivity. *Cis-trans* isomerism of enediol compounds has also been reported[7,8]. The disodium salt of DKG was synthesized from L-ascorbic acid by SeO_2 oxidation followed by addition of 2.5 equiv. of NaOH in methanol. The analytical values of the resulting yellow precipitate agreed with the formula of $C_6H_6O_7Na_2 \cdot H_2O$. Rapid decarboxylation was observed without a lag when this

Abbreviations: DAA, dehydro-L-ascorbic acid; DKG, 2,3-diketo-L-gulonic acid; DEAE-, dimethylaminoethyl-.

disodium DKG was used as a substrate, contrary to DKG which showed a 1-min lag. The sharp decrease in activity in acid conditions (pH 6.2) and the high apparent K_m ($1.5 \times 10^{-2} M$) also supported the presence of a enediol intermediate.



Department of Biochemistry, Faculty of Medicine, University of Tokyo, Tokyo (Japan)

YASUO KAGAWA
YOSHITAKE MANO
NORIO SHIMAZONO

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Cytochrome-like haemoproteins in the gut fluids of molluscs

Helicorubin, a haemoprotein of unknown physiological function, has been found in the gut fluids of certain molluscs, polychaetes and crustaceans[1–3], and some of its physical and biochemical properties have recently been characterized with purified preparations[4,5]. Except for erythrocruorin and chlorocruorin occurring in the blood of invertebrates, helicorubin is the only haemoprotein found extracellular in high concentrations.

Abbreviation: DEAE, diethylaminoethyl-.