React. Kinet. Catal. Lett., Vol. 34, No. 1, 179-184 (1987)

KINETICS AND MECHANISM OF THE OXIDATION OF L-ASCORBIC ACID BY 2,6-DICHLOROPHENOL-INDOPHENOL IN AQUEOUS SOLUTION

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Received January 2, 1986
Accepted September 4, 1986

The kinetics of oxidation of L-ascorbic acid by 2,6-dichlorophenolindophenol in aqueous solution has been studied. The rate of the reaction decreases with increasing pH since the hydrogen ascorbate ion is less reactive than the unionized L-ascorbic acid. The rate constants for the oxidation of the two species have been evaluated and a plausible mechanism of the reaction is suggested.

Исследована кинетика окисления L-аскорбиновой кислоты с помощью 2,6-дихлорфенол-индофенол в водных растворах. Скорость реакции уменьшается с увеличением pH, так как ион неионизированного аскорбата менее реактивен, чем неионизированная L-аскорбиновая кислота. Рассчитаны константы скорости окисления двух частиц и предложен вероятный механизм реакции.

INTRODUCTION

L-Ascorbic acid (LA) is an ene-diol γ-lactone in which the enolic hydroxy groups have strong reducing properties [1]. The reducing action of LA is important in several biological reactions such as the hydroxylation of proline and lysine in collagen [2]. LA can be quantitatively estimated by oxidizing

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agents in the presence of redox indicators or by electro-chemical methods [3-6].

Interestingly, 2,6-dichlorophenol-indophenol (DPIP) has been used not only as a redox indicator during the volumetric estimation of LA, but also for the quantitative oxidation of LA in biochemical systems [7-9]. For evaluating performance of two models of stopped-flow equipment, Benichiro et al. [10] studied the reduction of potassium ferricyanide and DPIP as test reactions and measured pseudo-first order rate constants at large concentration of LA. They observed that the rate constant for the latter reaction decreased when the pH was increased from 2.0 to 8.0 and the results were nonreproducible at high pH values. Hence a study of the kinetics of the reaction for evaluating the true order, rate constant and related kinetic parameters with the objective of formulating a plausible mechanism is necessary.

EXPERIMENTAL

Materials. LA and DPIP of high purity from Fluka A.G. and analytical grade chemicals from BDH were used for the preparation and standardization of the reactants. The ionic strength of the reaction medium was maintained at 0.05 M by potassium chloride. Since the LA versus DPIP titrations are conventionally carried out in phosphoric acid solutions, in the present work phosphate buffer was used to maintain the pH of the reaction medium.

Absorption spectra. DPIP has two distinct absorption spectra, one at pH's lower than 5.0 and the other at pH greater than 5.0. The molar absorptivities in these two pH ranges were determined to be 8.42×10^3 $M^{-1}cm^{-1}$ at λ_{max}=520 nm and 11.0×10^3 $M^{-1}cm^{-1}$ at λ_{max}=600 nm, respectively.

Kinetic study. A Shimadzu Recording UV-Visible Spectrophotometer Model UV-300 with Rapid Mixing Attachment RMA-1A was used for the kinetic study. Equal volumes of the reactants of identical concentrations were rapidly mixed and the absorbance was monitored at λ_{max}. The output of the spectrophotom-

eter was fed to an Aplab 15 MHz Cathode Ray Oscilloscope with calibrated time base. The CRO pattern was photographed by a Leica camera.

From the absorbance versus time plot thus recorded, the concentration of unreacted DPIP at various instants was evaluated. From the results 1 / [DPIP] was plotted versus time and the curve was satisfactorily linear, showing that the reaction was of the second order. The slope of this line, determined by a least squares analysis, was the rate constant.

Several repeated measurements yielded rate constants agreeing with one another within ± 3 %.

The rate constants were determined at various temperatures in the range of 20.0 to 35.0 °C and from the results the activation parameters evaluated (Table 1). Further, the kinetics were also studied at various pH's in the range of 3.0 to 6.0 (Table 2).

Table 1

Kinetic parameters of the oxidation of L-ascorbic acid by 2,6-dichlorophenol-indophenol at 25.0 °C

pH	Rate constant $k_2 (10^4 M^{-1} s^{-1})$	Energy of activation E_a (kJ mol^{-1})	Frequency factor $A(10^{11} M^{-1} s^{-1})$	Entropy of activation $S^{\neq}$ (JK^{-1} mol^{-1})
4.0	3.65	36.8	1.02	-42.5
6.0	0.661	38.3	0.339	-51.5

RESULTS AND DISCUSSION

It is interesting that the rate constant decreases appreciably with increasing the pH of the medium. LA ionizes in two steps [12], with pK values of 4.10 and 11.4. The second ionization constant is so small that the concentration of the di-ascorbate ion is insignificant in the pH range considered here. Rather, the relative concentrations of unionized LA and hy-

Table 2

Variation of rate constant of the oxidation of L-ascorbic acid by 2,6-dichlorophenol-indophenol with pH at 25.0 °C

pH	Rate constant $k_2(10^4 M^{-1}s^{-1})$	Relative concentration of unionized L-ascorbic acid	Relative concentration of hydrogen ascorbate ion
3.0	6.64	0.916	0.084
4.0	3.65	0.520	0.480
5.0	1.76	0.100	0.900
6.0	0.66	0.010	0.990

drogen ascorbate ion determine the observed rate constant at any particular pH. From the results at pH=4.0 and 5.0, the rate constants for the reaction of these species with DPIP can be estimated to be 5.8×10^4 $M^{-1}s^{-1}$ and 1.3×10^4 $M^{-1}s^{-1}$, respectively. An evaluation of these rate constants from data at either pH= =3.0 or 6.0 involves large errors due to the low relative concentration of either the hydrogen ascorbate ion or unionized LA.

From the above observations the following mechanism appears to be the most probable:

Due to the large electronegativity of the O and Cl atoms in DPIP, there is negative charge density ($-\delta$) on the O atom and positive charge density ($+\delta$) on the N atom. This dipole induces a positive charge ($+\delta$) on the more acidic H atom at the C_3 position and negative charge ($-\delta'$) on the other H atom of the ene-diol structure. Hence the formation of the activated complex is facilitated. In contrast, in the hydrogen ascorbate ion the induced polarization is less because of the negative charge and thereby the activated complex is formed less readily. Hence hydrogen ascorbate ion is less reactive than unionized LA. The formation of the activated complex from LA or hydrogen ascorbate ion and DPIP is the rate-

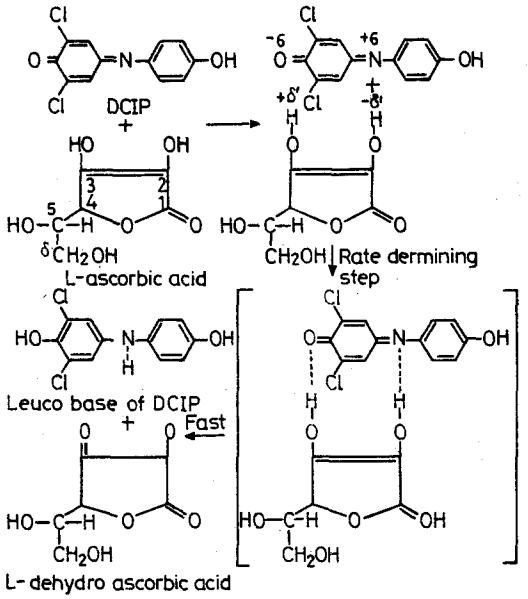

Fig. 1. Mechanism of the oxidation of L-ascorbic acid by 2,6-dichlorophenol-indophenol

determining step and this accounts for the observed second order of the reaction.

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