

THE KINETICS OF PARTIAL PROCESSES IN THE OXIDATION OF ASCORBIC ACID CATALYZED BY VANADYL TETRASULPHOPHTHALOCYANINE

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The rapid partial reactions of the oxidation of ascorbic acid by dioxygen with vanadyl tetrasulphophthalocyanine as a catalyst were studied by the stopped-flow method. The experimental data were treated on a computer and compared with the kinetic implications resulting from the proposed mechanism. Application of the adaptive identification method led to quantitative solution of the mechanism, *i.e.* determination of the values of all the isolated rate constants of the reaction mechanism.

In recent years, a number of works on the reactions of dioxygen have indicated that these reactions proceed by a coordination mechanism and that a number of kinetic characteristics are common for reactions catalyzed by metal complexes and by enzymes¹⁻³. The rate-controlling step is mostly the dissociation of the intermediate — the ternary complex between the catalyst (enzyme) and the substrate or substrates — preceded by a system of relatively rapid equilibria. In most kinetic studies, these preceding reactions are characterized by equilibrium constants.

Study of the rapid partial reactions of the oxidation of ascorbic acid by dioxygen catalyzed by vanadyl tetrasulphophthalocyanine (VOTSP) and cobalt tetrasulphophthalocyanine (CoTSP) provided a basis for proof of the existence of reactive intermediates in the reaction of the type of ternary complexes, $[O_2 \cdot V(III)TSP \cdot \text{ascorbic acid}]$ and $[O_2 \cdot CoTSP \cdot \text{ascorbic acid}]$ ^{3,4}.

In this work, experimental data on the rapid partial reactions were treated on a computer in order to give a quantitative solution for the reaction system, *i.e.* in order to determine all the rate constants of the elementary steps. The experimental data describe the kinetics of the changes in the individual forms of the catalyst and its adducts with the reactants; the concentrations of the free reactants, ascorbic acid and oxygen, were not measured.

EXPERIMENTAL

The tetrasodium salt of vanadyl tetrasulphophthalocyanine was prepared and purified by the procedure described earlier⁵. All the chemicals used were of *p.a.* purity. The stock ascorbic acid solutions (Farmakon, Czechoslovakia, Index Pharm. 3) were prepared fresh each other day and were stored in the dark at a temperature of 5°C.

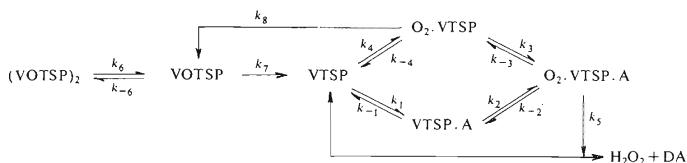
Kinetic measurements were carried out using a Durrum D-100 Stopped-Flow spectrophotometer, using 2 cm cuvettes and a mixed solution volume ratio of 1 : 1. The reaction was usually initiated by a change in the pH and in some cases by mixing a solution saturated with oxygen with an anaerobic solution of the other components at the same pH. The initial oxygen concentration ($1.25 \cdot 10^{-4}$ mol/l) equalled half the concentration of oxygen in the stock solution, given by its solubility at normal atmospheric pressure. The solution in the second stock syringe, usually containing ascorbic acid, was always freed of oxygen by bubbling with purified nitrogen. The reaction was carried out in 0.2M NaOH at 25°C. The catalyst concentration was $2.5 \cdot 10^{-6}$ mol/l. The ascorbic acid concentration was constant during the reaction and equalled $1 \cdot 10^{-2}$ mol/l.

The curves of the dependence of the absorbance on time were measured at three wavelengths, 655, 610 and 565 nm, corresponding to the absorption bands of the principal components of the reaction mixture. The curves were recorded at various time resolution values from $5 \cdot 10^{-3}$ to 5 s/cm. The calculation was carried out on the basis of the total kinetic curve with nonequidistant time points at a given wavelength, composed of the experimental curves recorded under identical reaction conditions at different time resolution values. Each total kinetic curve was characterized by 80 pairs of absorbance-time values. The molar absorption coefficient values for the individual components of the reaction mixture were determined from the static absorption spectra recorded under conditions where the concentration of the measured component was maximal. For the intermediates, a binary adduct between the catalyst and ascorbic acid (VTSP.A) and the ternary complex (O_2 .VTSP.A), a correction was made for the contributions from the other components to the absorbance at the given wavelength. The values of the molar absorption coefficients of the monomer VOTSP and vanadium tetrasulphophthalocyanine, VTSP, were taken from the literature or approximated on the basis of the literature data^{5,6}. The values for the assumed unstable adduct with dioxygen O_2 .VTSP were estimated within an order of magnitude.

RESULTS AND DISCUSSION

Model

The mechanism of oxidation of ascorbic acid by dioxygen catalyzed by VOTSP was described in an earlier work by reaction Scheme 1 and qualitative agreement was found with kinetic and non-kinetic (spectrophotometric) experiments³. The reduction of vanadyl tetrasulphophthalocyanine (VOTSP) to vanadium(III) tetrasulphophthalocyanine (VTSP) was demonstrated by EPR spectra⁷. It also follows from the EPR measurements that, after decomposition of the ternary complex, the catalyst is regenerated in the lower oxidation state (reaction designated k_5) and that the catalyst is reoxidized to the monomer VOTSP (reaction designated k_8).



SCHEME 1

A designates ascorbic acid and DA, dehydroascorbic acid. The individual components of the reaction system are either known ((VOTSP)₂, VOTSP, VTSP) or their existence has been proved (VTSP.A, O₂.VTSP.A); only the existence of the unstable adduct with oxygen, O₂.VTSP, is assumed. The wavelengths at which the curves of the dependence of the absorbance on time were recorded correspond to the absorption bands of the principal components of the reaction system – the dimer (VOTSP)₂ ($\lambda = 655$ nm), the binary adduct with ascorbic acid VTSP.A ($\lambda = 565$ nm) and the ternary complex O₂.VTSP.A ($\lambda = 610$ nm). Under the given experimental conditions, the other components of the reaction system do not yield observable separate maxima. The molar absorption coefficients of the individual components of the reaction mixture for the wavelengths given above are summarized in Table I.

The reaction kinetics were studied in two series of experiments; in the first series, the initial form of the catalyst was the dimer (VOTSP)₂, in the second series it was the binary adduct with ascorbic acid VTSP.A. Reactions designated k_5 , k_7 and k_8 are not, of course, elementary reactions, but their individual steps cannot be distin-

TABLE I

The molar absorption coefficients of components of the reaction system

Component	$E, \text{l mol}^{-1} \text{cm}^{-1}$		
	$\lambda = 655$ nm	$\lambda = 610$ nm	$\lambda = 565$ nm
(VOTSP) ₂	$2.7 \cdot 10^5$	$2.5 \cdot 10^4$	$3.0 \cdot 10^3$
VOTSP	$4.4 \cdot 10^4$ ^a	$5 \cdot 10^3$ ^b	$1 \cdot 10^3$ ^b
VTSP	$3.5 \cdot 10^2$ ^c	$9 \cdot 10^2$ ^c	$2 \cdot 10^3$ ^c
VTSP.A	$7.9 \cdot 10^3$	$1.5 \cdot 10^4$	$4.0 \cdot 10^4$
O ₂ .VTSP	10^2 ^d	10^3 ^d	$5 \cdot 10^3$ ^d
O ₂ .VTSP.A	$3.6 \cdot 10^4$	$9.3 \cdot 10^4$	$3.6 \cdot 10^4$

^a Ref.⁵; ^b approximation based on ref.⁵; ^c approximation based on ref.⁶; ^d estimate within one order.

guished by the method used. Thus constants k_5 , k_7 and k_8 have the significance of overall rate constants or of rate constants for the process controlling the rate of the partial reaction sequence.

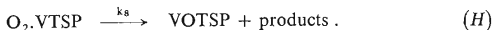
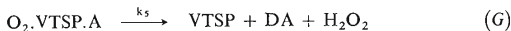
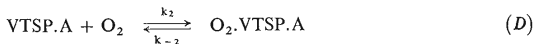
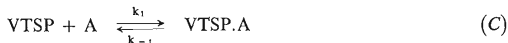
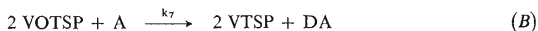
The mathematical equations describing the behaviour of the reaction system have been derived on the basis of the dependence of an additive property of the system, the absorbance, on time. It clearly follows that

$$A_i = 1\{E_{i1}[(\text{VOTSP})_2] + E_{i2}[\text{VOTSP}] + E_{i3}[\text{VTSP}] + E_{i4}[\text{VTSP.A}] + E_{i5}[\text{O}_2.\text{VTSP}] + E_{i6}[\text{O}_2.\text{VTSP.A}]\} \quad (1)$$

and

$$\frac{dA_i}{dt} = 1 \left\{ E_{i1} \frac{d[(\text{VOTSP})_2]}{dt} + E_{i2} \frac{d[\text{VOTSP}]}{dt} + E_{i3} \frac{d[\text{VTSP}]}{dt} + E_{i4} \frac{d[\text{VTSP.A}]}{dt} + E_{i5} \frac{d[\text{O}_2.\text{VTSP}]}{dt} + E_{i6} \frac{d[\text{O}_2.\text{VTSP.A}]}{dt} \right\}, \quad (2)$$

where A_i is the absorbance at the i -th wavelength, E_{ij} are the molar absorption coefficients of the j -th component and l is the thickness of the absorbing layer. The individual partial reactions in the mechanism given above can be expressed by the equations



It thus follows that the time dependences of the concentrations of the individual components are given by the equations

$$\frac{d[(\text{VOTSP})_2]}{dt} = -k_6[(\text{VOTSP})_2] + k_{-6}[\text{VOTSP}]^2 \quad (3)$$

$$\begin{aligned} \frac{d[\text{VOTSP}]}{dt} = & 2k_6[(\text{VOTSP})_2] - 2k_{-6}[\text{VOTSP}]^2 - 2k_7[\text{VOTSP}]^2 [\text{A}] + \\ & + k_8[\text{O}_2 \cdot \text{VTSP}] \end{aligned} \quad (4)$$

$$\begin{aligned} \frac{d[\text{VTSP}]}{dt} = & 2k_7[\text{VOTSP}]^2 [\text{A}] - k_1[\text{VTSP}] [\text{A}] + k_{-1}[\text{VTSP} \cdot \text{A}] - \\ & - k_4[\text{VTSP}] [\text{O}_2] + k_{-4}[\text{O}_2 \cdot \text{VTSP}] + k_5[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] \end{aligned} \quad (5)$$

$$\begin{aligned} \frac{d[\text{VTSP} \cdot \text{A}]}{dt} = & k_1[\text{VTSP}] [\text{A}] - k_{-1}[\text{VTSP} \cdot \text{A}] - k_2[\text{VTSP} \cdot \text{A}] [\text{O}_2] + \\ & + k_{-2}[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] \end{aligned} \quad (6)$$

$$\begin{aligned} \frac{d[\text{O}_2 \cdot \text{VTSP}]}{dt} = & k_4[\text{VTSP}] [\text{O}_2] - k_{-4}[\text{O}_2 \cdot \text{VTSP}] - k_3[\text{O}_2 \cdot \text{VTSP}] [\text{A}] + \\ & + k_{-3}[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] - k_8[\text{O}_2 \cdot \text{VTSP}] \end{aligned} \quad (7)$$

$$\begin{aligned} \frac{d[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}]}{dt} = & k_2[\text{VTSP} \cdot \text{A}] [\text{O}_2] - k_{-2}[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] + k_3[\text{O}_2 \cdot \text{VTSP}] [\text{A}] - \\ & - k_{-3}[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] - k_5[\text{O}_2 \cdot \text{VTSP} \cdot \text{A}] \end{aligned} \quad (8)$$

It also holds that $[\text{A}] = \text{constant}$ and the material balance gives

$$\begin{aligned} [\text{VOTSP}]_0 = & 2[(\text{VOTSP})_2] + [\text{VOTSP}] + [\text{VTSP}] + [\text{VTSP} \cdot \text{A}] + \\ & + [\text{O}_2 \cdot \text{VTSP}] + [\text{O}_2 \cdot \text{VTSP} \cdot \text{A}], \end{aligned} \quad (9)$$

where $[\text{VOTSP}]_0$ is the analytical concentration of the catalyst without reference to the oxidation state. The initial conditions for $t = 0$ in the first set of experiments are $[\text{VOTSP}]_0 = 2[(\text{VOTSP})_2]$; in the second series of experiments, it holds that $[\text{VOTSP}]_0 = [\text{VTSP} \cdot \text{A}]$; however, further results indicate that this assumption is not completely fulfilled.

The estimated values of the rate constants were used as initial values in the numerical computation. As the reaction system contains ascorbic acid in excess over the

catalyst and oxygen, and at the beginning of the reaction oxygen in excess over the catalyst, most partial reactions are monomolecular (dissociation of the dimer, of the adduct, of the ternary complex) or pseudomonomolecular. Thus the rate constants can be estimated on the basis of the half-lives, measured on sections of the kinetic curve where it can be assumed that the course is determined primarily by a single reaction. The other rate constants were estimated within one order of magnitude and the rate constants for monomerization and dimerization were taken from the literature⁵. The estimated rate constants are given in Table II.

Evaluation of the Rate Constants

The mathematical model describing the reaction scheme contains 8 variables and 13 unknown parameters — the rate constants characterizing the individual reactions. The variables are the concentrations of the individual components. The concentrations are not explicitly known but implicitly as the dependence of the absorbance on time. The experimental data (absorbance at time t) are of integral nature, *i.e.* they are the solution of a set of differential equations describing the reaction course.

It is evident from the estimates of the kinetic parameters given in Table II that the numerical constant values differ by several orders of magnitude. It is known that, in numerical solution of a set differential equations describing dynamic changes

TABLE II
Rate constants of the partial reactions

k_i	Initial estimate	Calculated value	Dimensions
k_1	$3 \cdot 10^3$	$1 \cdot 11 \cdot 10^3$	$l \text{ mol}^{-1} \text{ s}^{-1}$
k_{-1}	10	$2 \cdot 83 \cdot 10^3$	s^{-1}
k_2	$2 \cdot 10^6$	$8 \cdot 21 \cdot 10^5$	$l \text{ mol}^{-1} \text{ s}^{-1}$
k_{-2}	10^4	$2 \cdot 26 \cdot 10^4$	s^{-1}
k_3	10^3	$1 \cdot 45 \cdot 10^3$	$l \text{ mol}^{-1} \text{ s}^{-1}$
k_{-3}	10^2	$3 \cdot 37 \cdot 10^3$	s^{-1}
k_4	10^4	$2 \cdot 45 \cdot 10^2$	$l \text{ mol}^{-1} \text{ s}^{-1}$
k_{-4}	10^2	$1 \cdot 36 \cdot 10^3$	s^{-1}
k_5	$7 \cdot 10^{-2}$	$2 \cdot 08 \cdot 10^2$	s^{-1}
k_6^a	$4 \cdot 5 \pm 0 \cdot 2$	$1 \cdot 89 \cdot 10^3$	s^{-1}
k_{-6}^a	$(8 \cdot 4 \pm 0 \cdot 8) \cdot 10^6$	$1 \cdot 52 \cdot 10^2$	$l \text{ mol}^{-1} \text{ s}^{-1}$
k_7	10^3	$6 \cdot 23 \cdot 10^2$	$l^2 \text{ mol}^{-2} \text{ s}^{-1}$
k_8	10^4	$1 \cdot 12 \cdot 10^3$	s^{-1}

^a Ref.⁵.

in a chemical system using standard methods intended for automatic computer integration, the computation is unreasonably long and "numerical explosion" can even occur. Sets of ordinary differential equations exhibiting this type of behaviour are designated as "stiff" systems⁸. In their solution it is not possible to unambiguously decide on the use of a particular method on the basis of the literature and it is necessary to solve the problem by empirical testing of a whole group of methods programmed in a similar manner and arranged in a polyalgorithm⁹.

It is not possible to simplify the set of differential equations using the pseudo-steady-state approximation because of the character of the experiment — the kinetics of rapid partial reactions were studied. In addition, the size of the set of differential equations makes it impossible to estimate the effect of this procedure on the final result^{10,11}. Consequently, the kinetic processes were simulated using a full, unreduced set of equations. A further complication, compared with the use of standard methods of analysis of kinetic data, occurs in the determination of the unknown parameters as a result of the fact that the changes in the concentrations of the individual components are not explicitly known.

It was found that the set of differential equations given in this work can relatively best be solved using the semi-implicit Runge-Kutta method¹² chosen, using the STIFFSOLVER 80 program, from a set of about 200 programmed methods¹³. This method is very effective; nonetheless, solution of the given set of equations during numerical simulation of changes in the reaction system required about 7 min of CPU time per integration on the IBM 370/135 computer.

Use of the quasilinearization method¹⁴ was not found to be useful because of difficulties involved in simultaneous integration of the sensitivity equations (as the "stiff" parameters of the system of differential equations become worse).

It is possible to minimize differences between the calculated and measured absorbance values using a not too effective method¹⁵ chosen from the group of direct search methods, but a single integration of the differential equations for 3 different initial experimental conditions take about 15 minutes. For 13 unknowns and 300 to 500 iterations necessary for finding the minimum, the computation would require about 100 CPU hours on a computer comparable with the IBM 370/135 instrument.

Because of the unsuitability of the tested methods for determining unknown kinetic parameters from the integral data attention was turned to the use of unconventional methods for identification of the differential systems, derived from the optimal control methods¹⁵, sometimes also called adaptive identification methods. In these methods, the minimal difference between the experimental data and the kinetic model is optimized and the controlling quantity is the vector of the unknown kinetic parameters. Here we used the variant of the gradient minimization method so that, in addition to a system describing the reaction kinetics, a set of differential equations adapting the vector of the unknown parameters to the experiment according to the following

equation was solved:

$$\frac{dk_i}{dt} = -\alpha \operatorname{grad} \left(\sum_{k=1}^3 \Delta_k^2 \right), \quad (10)$$

where k is the i -th rate constant, Δ_k is the difference between the absorbances measured and calculated for all three wavelengths simultaneously and α is the damping constant of the method (0.2–0.7).

Consequently, during a single integration, the vector of the unknown rate constants is obtained, where it is also possible to estimate the errors in the parameters resulting from changes in the studied parameter along the trajectory limited by the experimental data. A detailed description of the determination of unknown parameters by the method of adaptive identification can be found in the literature^{15,16}. The values of the kinetic parameters were refined in the final stage of the work using the method of nonlinear regression so that all the experiments were treated simultaneously, yielding the average values given in Table II. As the adaptive method yielded a very good approximation, the iteration process was rapidly terminated.

The maximal possible errors in the parameters are estimated on the basis of the fact that the changes of concentration and of the values of the kinetic parameters (rate constants) k_i are implicitly given in the form

$$A = \sum E \cdot c(k_i), \quad (11)$$

where A is the absorbance and E is the molar absorption coefficient. After differentiation, the relationship between the differentials is obtained

$$dA = \sum dE \cdot c + \sum dc \cdot E, \quad (12)$$

$$dc = \sum_j^k (\partial c / \partial k) \cdot dk. \quad (13)$$

As functional assignment is usually given by the product of the kinetic parameter and the concentration, taking of logarithms and rearrangement of the relationship yield an estimate of the maximal error in the rate constant in the implicit form

$$\left| \frac{\Delta A}{A} \right| = \sum \left| \frac{\Delta E}{E} \right| + \sum \left| \frac{\partial c}{\partial k} \cdot \frac{\Delta k}{k} \right|, \quad (14)$$

where ΔA , ΔE and Δk are the relative errors. The relative error in absorbance calculation, is given by the sum of the relative errors in the kinetic parameters and molar absorption coefficients, multiplied by the parametric sensitivity. The estimate of the

maximal error obtained in this way is considerably overestimated compared to reality as mutual compensation of errors is not considered. The error in the individual absorbance measurement is about ± 0.001 ; the principal source of error is thus the imprecision in the determination of the molar absorption coefficients of the unstable intermediates. The maximal error in the individual kinetic parameters obtained by the estimation described above is not greater than 10%.

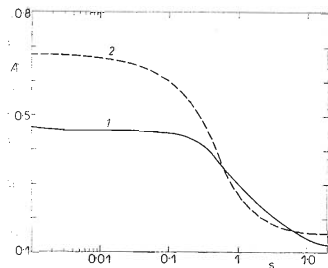


FIG. 1

Comparison of the experimental and simulated absorbance-time curves at $\lambda = 655$ nm. Main absorbing component $(\text{VOTSP})_2$; 0.2M-NaOH , initial concentration: $2.5 \cdot 10^{-6}\text{M-(VOTSP)}_2$; $1.0 \cdot 10^{-2}\text{M-ascorbic acid}$, $1.25 \cdot 10^{-4}\text{M-O}_2$. 1 experimental values, 2 simulated values

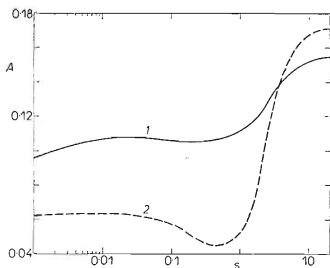


FIG. 2

Comparison of the experimental and simulated absorbance-time curves at $\lambda = 610$ nm. Main absorbing component $\text{O}_2\text{-VTSP.A}$; 0.2M-NaOH , initial concentrations: $2.5 \cdot 10^{-6}\text{M-(VOTSP)}_2$; $1.0 \cdot 10^{-2}\text{M-ascorbic acid}$, $1.25 \cdot 10^{-4}\text{M-O}_2$. 1 experimental values, 2 simulated values

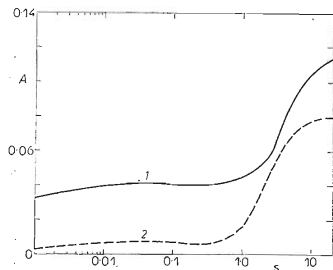


FIG. 3

Comparison of the experimental and simulated absorbance-time curve at $\lambda = 565$ nm. Main absorbing component VTSP.A ; 0.2M-NaOH , initial concentrations: $2.5 \cdot 10^{-6}\text{M-(VOTSP)}_2$, $1.0 \cdot 10^{-2}\text{M-ascorbic acid}$, $1.25 \cdot 10^{-4}\text{M-O}_2$. 1 experimental values, 2 simulated values

In Figs 1–3, the experimental kinetic curves are compared with the shape simulated by the computer on the basis of the determined rate constants for the first series of experiments, *i.e.* for experiments beginning with (VOTSP)₂. Because of the nature of the problem and atypical character of the formulation, as described in the above text, the agreement between the experiment and simulated results can be considered as satisfactory. The simulated curve has the correct shape and positions of the extremes, which are most important for solution of the kinetics. The shift in the absolute values of the absorbance for the experimental and simulated curves is a result of imprecision in the approximation of the molar absorption coefficients of the unstable reaction intermediates. Consequently, the second series of experiments, beginning with the adduct VTSP.A, is also accompanied by a much larger error; in addition, it is apparently practically impossible to fulfill the condition that all the catalyst at the beginning of the reaction be present as VTSP.A. The agreement between the simulated and experimental curve is then worse.

The lack of agreement between the calculated rate constants for the preceding monomer-dimer equilibrium, k_6 and k_{-6} , and the values given in the literature is marked⁵. If the preceding equilibrium were as immobile as suggested by the literature data, the reaction system could not behave as observed.

The largest rate constant in the reaction system is constant k_2 , corresponding to bonding of dioxygen to the binary adduct VTSP.A. The bonding of dioxygen directly to the catalyst has a much lower rate constant (k_4). This is in agreement with the earlier observation¹⁷ that the bonding of dioxygen to the complex is much faster if the substrate, *i.e.* substance with nucleophilic properties, is bonded in the *trans*-position. This phenomenon could be denoted as a kinetic discriminatory effect, in contrast to thermodynamic discriminatory effects¹⁸, where, *e.g.*, the nucleophilic properties of the ligand in the *trans*-position increase the strength of the dioxygen bond¹⁹.

The methods derived from the theory of optimal control have not yet been used in this form to solve the mathematical model of the reaction system, *i.e.* to determine the kinetic parameters. Classical methods for the analysis of kinetic data for complicated mechanisms cannot be used with this formulation of the problem. It appears that combination of the adaptive method with classical non-linear regression is suitable for solution of complicated reaction systems.

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