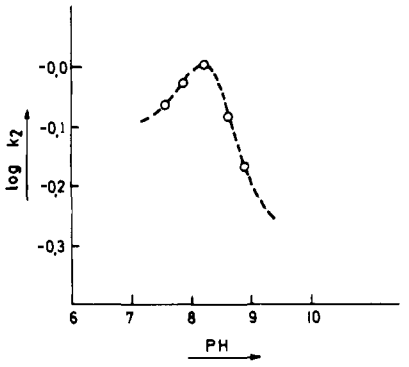

Figure 1. pH dependence of the second-order rate constant of the reaction between V^{2+}(aq) and cysteine. The reaction was followed by stopped flow at 400 nm; $[V^{2+}] = 3 \times 10^{-4}$ M, [cys] = 10^{-1} M; room temperature.

for substitution on $V(H_2O)_6^{2+}$.[4] The preexponential factor is 2.1×10^6.

The formation of dihydrogen follows linear first-order kinetics with respect to V^{II}–cys. The value of the rate constant at 21 °C is 2.3×10^{-3} s^{-1}. The estimation was based on dihydrogen and the known stoichiometry (1:0.5). The rate of this stage is independent of the concentrations of cysteine and of pH (in the range 7.5–8.5; Table B, supplementary material). The Arrhenius energy of activation is 12.9 ± 0.4 kcal mol^{-1} (Figure B_1, supplementary material). The preexponential factor is 5×10^6.

Products of oxidation and/or reduction of cysteine itself were not detected.

The lack of dependence of the rate of the redox reaction on hydrogen and/or hydroxide ion concentration (10-fold change around pH 8) forces us to rule out separate implication of these ions in the rate-determining step and to suggest that both are involved simultaneously or that neutral water is the reactant. One way both H^+ and HO^- could be implicated is to assume that the form of the complex participating in the rate-determining step of the redox process is hydrolyzed and that this step is catalyzed by acid.

Addition of V^{IV} or cystine, after V^{II}–cys is formed, supresses dihydrogen formation, the product in this case being only V^{III}–cys.

Both V^{II}–cys and V^{III}–cys are intensely yellow. In Figure 2 we compare their spectra with those of $V(H_2O)_6^{2+}$ and $V(H_2O)_6^{3+}$. The main feature is the appearance of two new charge-transfer bands in the UV region, one of which (ca. 280 nm) can be assigned to ligand-to-metal charge transfer. Similar assignments have been made[5] for a variety of other complexes containing metal-to-sulfur bonds. The other band (ca. 390 nm) is tentatively assigned to a metal-to-ligand charge transfer and indicates strong back-bonding. In fact, with mercaptoacetate this back-bonding is so strong that it leads to a net electron transfer from V^{II} to the ligand and to a break of the C–S bond.[6]

The increase in absorptivities and the broadening and the small shifts of the d–d transitions are consistent with the postulate of strong interaction between sulfur and the metal ions.

Spectra of the V^{II}–cys complex could not be taken under conditions of fast hydrogen production (because V^{II}–cys changes quickly to V^{III}–cys and H_2 bubbles form). The V^{III}–cys spectrum, however, did not change appreciably for pH values in the range 7.0–8.5 and for all ratios [cys]/[V^{III}] tried down to the value of 20.

Thus, it seems that the extra electron in the V^{II}–cys system remains delocalized over V^{II} and cysteine, until it is transferred

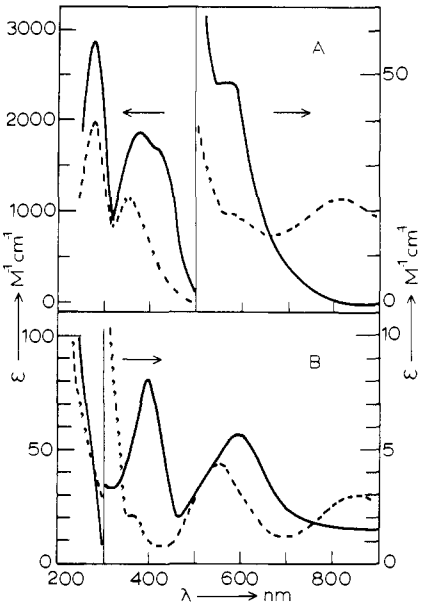

Figure 2. (A) Spectra of V^{II}–cys (---) and V^{III}–cys (—) complexes at pH 8, room temperature; $[V^{II}] = [V^{III}] = 9 \times 10^{-4}$ M, [cys] = 9.4×10^{-2} M. (B) The corresponding spectra for $V(H_2O)_6^{2+}$ and $V(H_2O)_6^{3+}$. The spectra in A were taken with cysteine in the reference compartment.

to an oxidant such as V^{IV} or cystine or, by a slower process, to a water molecule. In the absence of oxidants and/or water the electron can presumably be "stored" indefinitely.

Registry No. Vanadium, 7440-62-2; L-cysteine, 52-90-4; water, 7732-18-5.

Supplementary Material Available: Tables A and B, containing experimental data for the rate law of the substitution reaction and experimental data for the stoichiometry and the rate law of the redox step, Figures A_1 and A_2, showing kinetic and Arrhenius plots, respectively, for the substitution reaction, and Figure B_1, showing the Arrhenius plot for the redox reaction (4 pages). Ordering information is given on any current masthead page.

Phenomenon of Slowing Down in the Autocatalytic Trypsinogen to Trypsin Conversion in a Continuous-Flow Stirred-Tank Reactor

H. Lachmann and F. W. Schneider*

Institute of Physical Chemistry, University of Wuerzburg D-8700 Wuerzburg, GFR

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The phenomenon of critical slowing down is an important feature of open nonlinear chemical reactions near points of instability.[1-4] It signifies that the approach to a steady state is very slow near these points. Critical slowing down was predicted theoretically by Schlögl[5] for certain open autocatalytic systems. In recent theoretical work we showed that critical slowing down may occur when a simple autocatalytic reaction A + B → 2B is carried out in a continuous-flow stirred tank reactor (CSTR).[6] Slowing down effects in a CSTR were also calculated by us in computer simulations of a cyclic mechanism of enzymatic poly

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(5) (a) Schmidtke, H. H. *Ber. Bunsenges. Phys. Chem.* **1967**, *71*, 1138. (b) Jorgensen, C. K. *Inorg. Chim. Acta Rev.* **1968**, *2*, 65. (c) Weschler, C. J.; Deutch, E. *Inorg. Chem.* **1973**, *12*, 2683. (d) Lane, R. H.; Sedor, F. A.; Girloy, M. J.; Eisenhart, P. F.; Bennett, J. P., Jr.; Ewall, R. X.; Bennett, L. E. *Ibid.* **1977**, *16*, 93. (e) Balahura, R. J.; Lewis, N. A. *Ibid.* **1977**, *16*, 2213.

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(1) H. Haken, "Synergetics. An Introduction", Springer, Berlin, 1977.

(2) F. Jähnig and P. H. Richter, *J. Chem. Phys.*, **64**, 4645 (1976).

(3) C. Vidal and A. Pacault, Eds., "Nonlinear Phenomena in Chemical Dynamics", Springer, Berlin, 1981, part IV.

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(5) F. Schlögl, *Z. Phys.*, **248**, 446 (1971); **253**, 147 (1972); *Ber. Bunsenges. Phys. Chem.*, **84**, 351 (1980).

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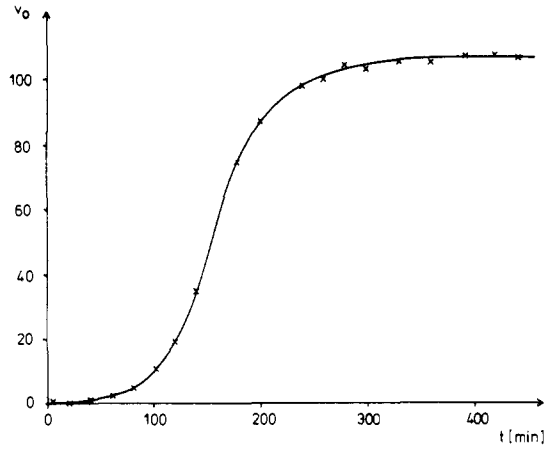

Figure 1. Autocatalytic conversion of Tg to Tr at pH 8, 25 °C, in the closed system; initial rate v_0 of L-BAPNA assay (arbitrary units) vs. time for $(Tr)_0 = 2.5 \times 10^{-7}$ M and $(Tg)_0 = 2.5 \times 10^{-5}$ M.

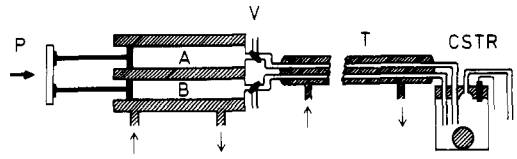

Figure 2. CSTR system: P = syringe pump; A, B = Hamilton syringes (20 mL) in a thermostated block; V = valves; T = supply tubes (thermostated); CSTR = reactor cell with thermistor and stirring bar.

A/poly U replication.[7] Molecular dynamics calculations of a three-step autocatalytic mechanism also showed a slowing-down effect even for a few hundred reacting molecules.[8]

In this work we report a biological example of slowing down in an isothermal CSTR,[9] namely, the time-honored autocatalytic conversion of trypsinogen (Tg) to trypsin (Tr) at pH 8, 25 °C, which was studied as early as 1936 in a conventional closed system by Kunitz and Northrop.[10] Since the Tg conversion cannot be followed with sufficient precision directly in the reaction mixture, we employed a separate spectrophotometric determination of Tr in the presence of Tg. This assay for Tr uses in separate aliquots 1×10^{-4} M *N*-benzoyl-L-arginine-*p*-nitroanilid (L-BAPNA),[11] which is hydrolyzed by Tr to *N*-benzoylarginine and *p*-nitroaniline in 0.1 M Tris·HCl buffer, pH 8, 25 °C, containing 0.05 M $CaCl_2$. In each aliquot the appearance of *p*-nitroaniline is followed at 405 nm as a function of time, and the initial rate of the absorbance–time curve is determined. This initial rate measurement turns out to be highly precise, and it can be shown to be a linear function of the Tr concentration from 8×10^{-6} to 8×10^{-9} M Tr. An example of the kinetics in the closed system is given in Figure 1 for a low initial Tr concentration ($(Tr)_0 = 2.5 \times 10^{-7}$ M) and a high Tg concentration ($(Tg)_0 = 2.5 \times 10^{-5}$ M) at pH 8 and 25 °C. The experiment shows the typical autocatalytic conversion that may be described by the overall phenomenological mechanism[10]

$$Tg + Tr \xrightarrow{k_1} 2Tr \text{ (+hexapeptide)} \qquad (1)$$

For the study of the open kinetics and the slowing down effect we used the CSTR[12] described in Figure 2. It consists of a 1-cm

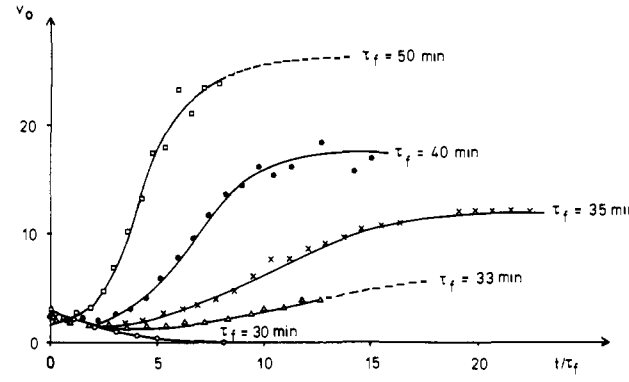

Figure 3. Autocatalytic conversion of Tg to Tr at pH 8, 25 °C, in the CSTR; initial rate v_0 of L-BAPNA assay (arbitrary units) vs. units of residence time ($=t/\tau_f$) for $(Tr)_0 = 1.25 \times 10^{-6}$ M and $(Tg^0) = 2.5 \times 10^{-5}$ M at τ_f = 30, 33, 35, 40, and 50 min.

shortened fluorimetric cell of 1.74-mL volume capped with a Teflon stopper that contained three microtube ducts and a thermistor lead. The in-flow rate is controlled by a highly linear syringe pump (Infors Precidor) that uses two thermostated Hamilton syringes (20 mL). The CSTR was kinetically tested with dye solutions and found to behave "ideally", i.e., concentration and temperature gradients did not exist since complete mixing occurred in less than 3 s by efficient magnetic stirring at ~1200 rpm. Tg (in 10^{-3} M HCl) enters the reactor at a certain in-flow rate through tube A; tube B transports Tris·HCl buffer and 0.05 M $CaCl_2$ at pH 8 at the same flow rate. This arrangement is used since Tg is optimally stable in acidic solution. All parts of the CSTR system (reactor, supply tubes, syringes) are thermostated at 25.0 ± 0.1 °C. The effective reaction time represents an average over the time interval required to collect the sample, which varied between 60 and 90 s depending upon the flow rate.

A dramatic slowing down effect is observed at high flow rates, i.e., at short residence times τ_f (Figure 3). In fact the steady state of Tr has not even been reached after about 18 units of residence time (~600 min) have elapsed from the start of the flow experiment at τ_f = 33 min. For a "normal" reaction without any feedback, 95% of a steady-state concentration is always reached within ~3 units of residence time under comparable conditions. The reactor was initially filled with a Tr concentration of $(Tr)_0 = 1.25 \times 10^{-6}$ M in buffer. An *effective* Tg concentration of $(Tg^0) = 2.5 \times 10^{-5}$ M flowed into the CSTR at various flow rates. The results at high flow rates are in marked contrast to those at the lowest flow rate used, where the steady state is reached more rapidly, i.e., in about 8 units of residence time at τ_f = 50 min. Obviously higher flow rates lead to increased slowing down. The slowing-down effect is maximal at the "critical" flow rate. This occurs in the τ_f range between 30 and 33 min. The critical residence time is difficult to realize experimentally. An increase in the flow rate beyond the critical value causes the initial Tr to empty out of the reactor also with a slowing-down effect. Here the rate of out-flow is larger than the rate of Tr formation. All curves in Figure 3 show an early minimum on account of the physical out-flow.

It may be shown theoretically[6] that the phenomenon of critical slowing down is not affected by nonautocatalytic steps such as linear consecutive reactions, aggregation, etc. Literature work as well as our own experiments does not provide any evidence of aggregation of Tg or Tr under our experimental conditions.

A Jacobi matrix analysis[6] of eq 1 for small perturbations from the steady state yields two eigenvalues, $1/\tau_1 = k_f$ and $1/\tau_2 = |k_f - k_1(Tg^0)|$. It is interesting to note that τ_2 may formally approach infinity if $k_f = k_1(Tg^0)$. However, a small perturbation no longer disappears exponentially at the critical flow rate, but it decays[6] with $1/t$. Thus the expression obtained for an exponential relaxation time τ_2 is not defined at this particular point but only in its neighborhood.

Open systems that show slowing down may act as kinetic buffers since large exterior concentration perturbations may be

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(8) M. Heinrichs and F. W. Schneider, to be submitted for publication. M. Heinrichs, Dissertation, University of Wuerzburg, 1981.
(9) Presumably the first experimental slowing-down effect has been measured in enzymatic poly[d(A-T)] synthesis in the CSTR, although it was not recognized as such at the time (F. W. Schneider, *Biopolymers*, **15**, 1 (1976), Figure 4).
(10) M. Kunitz anmd J. H. Northrop, *J. Gen. Physiol.*, **19**, 991 (1936).
(11) H. Nakata and S. Ishii, *J. Biochem.*, **72**, 281 (1972).
(12) H. Lachmann, in "Nonlinear Phenomena in Chemical Dynamics", C. Vidal and A. Pacault, Eds., Springer, Berlin, 1981, p 266.

"neutralized" by the system. The general biological relevance of slowing down is presently an open question.

Acknowledgment. We thank H. Schraut for expert technical assistance and M. Heinrichs for stimulating discussions. We also thank the Fonds der Chemischen Industrie for partial support of this work.

Registry No. Trypsinogen, 9002-08-8; trypsin, 9002-07-7.

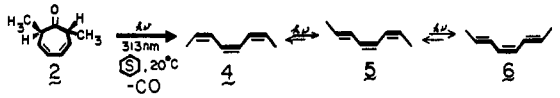
Stereospecific Photodecarbonylation of *cis*- and *trans*-2,7-Dimethyl-3,5-cycloheptadienone: Observation of an Apparent Symmetry-Forbidden Concerted Cheletropic Fragmentation[1]

David I. Schuster* and Li Wang

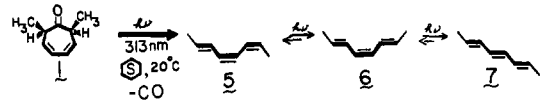
Department of Chemistry, New York University
New York, New York 10003

Received November 12, 1982

We have previously reported that photodecarbonylation of 3,5-cycloheptadienones occurs from a short-lived singlet electronic excited state of mixed electronic configuration[2,3] and speculated that the mechanism of this reaction involves simultaneous cleavage of both bonds to the carbonyl carbon, i.e., a concerted cheletropic fragmentation.[5,6] However, a stepwise mechanism invoking acyl-alkyl diradicals could not be excluded from consideration. We now report that the analogous reactions of *cis*- and *trans*-2,7-dimethyl-3,5-cycloheptadienone (**1** and **2**) are stereospecific and occur with conrotation at the methyl-bearing carbons to give initially (*Z,Z,E*)- and (*Z,Z,Z*)-2,4,6-octatriene, respectively, which then undergo further isomerization (see eq 1 and 2).



(1)



(2)

The synthesis of **1** and **2** from tropone will be described in detail elsewhere.[18] These compounds, purified by HPLC, readily isomerize thermally to 2,7-dimethyl-2,4-cycloheptadienone (**3**) under a variety of conditions but are stable on storage at low temperatures. The assignment of stereochemistry to **1** and **2** is based on NMR spectra of their Diels-Alder adducts with 4-phenyl-1,2,4-triazolin-3,5-dione and an X-ray structure of the adduct of *cis*-dienone **1**.[7]

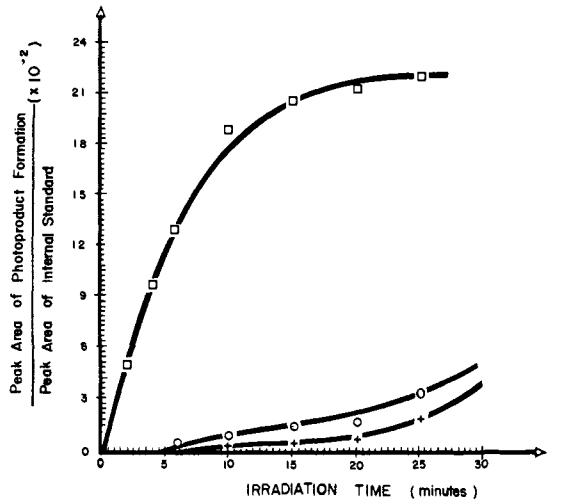


Figure 1. Irradiation of **2** (0.0610 M) in cyclohexane at 313 nm (20 °C). Photoproducts (*Z,Z,Z*)-, (*E,Z,Z*)-, and (*E,Z,E*)-2,4,6-octatriene (**4**-**6**, respectively) are represented by □, ○, and +, respectively.

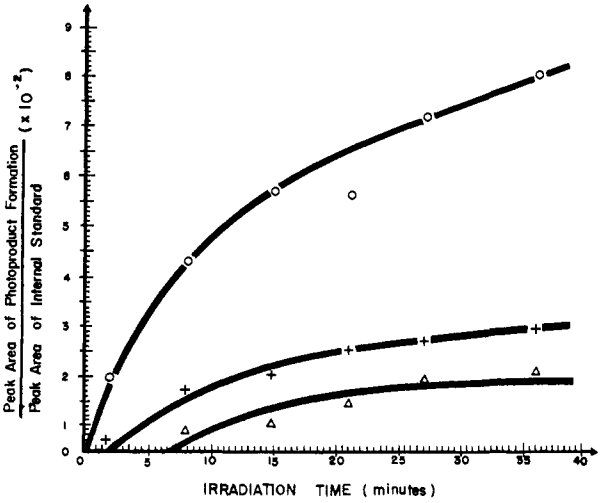


Figure 2. Irradiation of **1** (0.0690 M) in cyclohexane at 313 nm (20 °C). Photoproducts (*E,Z,Z*)-, (*E,Z,E*)-, and (*E,E,E*)-2,4,6-octatriene (**5**-**7**, respectively) are represented by ○, +, and △, respectively.

The three trienes **4**-**6** were independently synthesized according to the method of Marvell,[8] separated and purified by preparative GLC, and characterized by UV[8] and NMR spectroscopy. The all-trans *E,E,E* isomer **7**, mp 50-51 °C, was prepared by irradiation of the other trienes. The products of irradiation of **1** and **2** in cyclohexane at 313 nm were analyzed by GLC with an internal standard (*n*-tridecane) at various intervals of time, with the results as shown in Figures 1 and 2. No interconversion of dienones **1** and **2** was detected during the irradiations, thus excluding reversible formation of an acyl-akyl diradical that can undergo bond rotation prior to ring closure.[9]

These 3,5-cycloheptadienones are believed to possess a molecular structure with C_2 symmetry containing a twisted diene moiety.[2-4] In such a structure, the methyl groups of the *cis*-dienone **1** would occupy pseudoquatorial and pseudoaxial positions, while the methyls in the *trans*-dienone **2** are both either pseudoaxial or pseudoequatorial. Molecular mechanics calculations are in progress to determine which of the conformations of **2** is more

(1) Photochemistry of Ketones in Solution. 70. Part 69: Brisimitzakis, A. C.; Schuster, D. I. *Tetrahedron Lett.* **1982**, *44*, 4535.

(2) Schuster, D. I.; Sckolnick, B. R.; Lee, F.-T. H. *J. Am. Chem. Soc.* **1968**, *90*, 1300. Schuster, D. I.; Kim, C. W. *Ibid.* **1974**, *96*, 7437.

(3) INDO calculations indicate that the S_1 state of 3,5-cycloheptadienones with overall C_2 symmetry is highly mixed configurationally, with about 80% contribution from an n,π^* configuration. Extinction coefficients for absorption above 290 nm are greatly enhanced over those for corresponding α,β-enones or saturated ketones, as is typical for twisted β,γ-enones.[4] For a full discussion of the photochemistry and spectroscopy of these systems, see: Schuster, D. I.; Eriksen, J. *J. Org. Chem.* **1979**, *44*, 4254.

(4) For a review on the photochemistry and spectroscopy of β,γ-enones, see: Schuster, D. I. "Rearrangements in Ground and Excited States"; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 3, Essay 17. See also: Houk, K. N. *Chem. Rev.* **1976**, *76*, 1.

(5) Woodward, R. B.; Hoffmann, R. "The Conservation of Orbital Symmetry"; Verlag Chemie/Academic Press: New York, pp 152 ff.

(6) For a review of cheletropic reactions, see; Mock, W. L. In "Pericyclic Reactions"; Marchand, A. P., Lehr, R. E., Eds.; Academic Press: New York, 1977; Vol. II, pp 141-179.

(7) Studies carried out in collaboration with Professor J. M. van der Veen, Stevens Institute of Technology, to be published elsewhere.

(8) Schatz, B. S. Ph.D. Dissertation, Oregon State University, 1967. Marvell, E. N.; Caple, G.; Schatz, B. *Tetrahedron Lett.* **1965**, 385.

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