CHIMIA 1999, 53, No. 5

Chimia 53 (1999) 232–234

ISSN 0009–4293

Getting Slow-Motion Pictures of Fast Reactions

Jakob Wirz*

Abstract. We investigate reaction mechanisms by monitoring the kinetics of transient intermediates. The technique is flash photolysis, the main targets are diradicals, unstable tautomers (enols), and phototriggers for time-resolved biochemical studies.

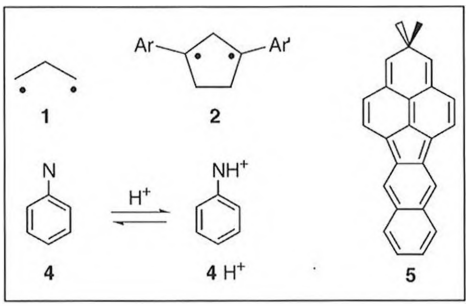
Jakob Wirz studied chemistry at the ETH Zürich and received his Ph.D. in 1970 from *E. Heilbronner*. He spent a post-doctoral period working with *G. Porter* (Royal Institution) and *D. Barton* (Imperial College) in 1970–1971. He completed his habilitation in 1978 in Basel and has been a Professor at the University of Basel since 1984. He received the annual prize from the Swiss Chemical Association in 1976 and the *Werner* Prize in 1982. Amongst his current duties, he is chairman of the European Photochemistry Association. Further details of his research interests are to be found at http://www.unibas.ch/photochemie/wirz.html.

Diradicals, Carbenes, Nitrenes

Chemistry is about fission and fusion of bonds. At the intermediate stages of reaction we are commonly faced with diradicals. Diradicals are molecules with a broken bond, two free valences and two unpaired electrons. The lowest electronic singlet and triplet states of such molecules are of comparable energy, but very different reactivity. The chameleon-like electronic structure of diradicals makes an anticipation of their chemical properties difficult. The goal of our studies is to obtain guidelines for the prediction of the multiplicity, stability and chemical reactivity to be expected from diradicals and insight into their responses to changes in manageable parameters such as temperature, solvent polarity, and substitution. Such development may assist recent efforts to make practical use of diradicals for synthetic or biochemical purposes, or as building blocks for organic materials with unusual magnetic and electric properties.

Singlet diradicals, such as **1**, have lifetimes on the femtosecond time scale. Triplet-state diradicals are relatively long-lived due to the spin barrier and are well-characterized. Kinetic studies of diradical intermediates and theoretical models to predict their rates of intersystem crossing have led to systematic structure-reactivity and multiplicity relationships for diradicals **2** [1]. We currently focus on singlet diradicals. 2,2-Difluoro-substitution of cyclopentane-1,3-diyl diradicals **2** reverses the effect of through-bond coupling and results in a remarkably persistent (τ = 80 ns), localized diradical with a singlet ground state [2]. The absorption spectrum and lifetime of singlet **4** were determined [3]. 2*H*-Dibenzo[*cd,k*]fluoranthene **5** is persistent at low temperature; it is the first *Kekulé* hydrocarbon with a triplet ground state; the low-lying singlet state of **5** is thermally populated [4].

Phenyl nitrene **4** and its conjugate acid **4**H$^+$ do not behave as a 'normal' acid-base pair: the rates of protonation of **4** and deprotonation of **4**H$^+$ in aqueous solution are substantially lower than generally found for proton transfer between heteroatoms. Theory predicts that *N*-protonation of singlet **4** is a symmetry-forbidden process [5].



Proton Transfer Involving Carbon Atoms: Keto-Enol Equilibria

Direct kinetic measurements of equilibrium reactions require that non-equilibrium mixtures can be generated in a time shorter than that needed to re-establish the equilibrium. Keto-enol equilibria in the ground state can be studied by flash photolysis if the unstable enols can be generated photochemically from suitable precursors [5]. Kinetic studies performed over the last two decades have provided absolute rate constants and reliable equilibrium constants for a broad range of prototropic reactions. The range of known kinetic and thermodynamic data on keto-enol protomeric equilibria now covers enolization constants varying by about 30 and rate constants by about 20 orders of magnitude (*Fig. 1*). The free-energy relationship between these quantities shows strong curvature, which defines the intrinsic barrier of proton transfer to the β-carbon atom of enols in terms of *Marcus*' theory of proton transfer, $\Delta G_0^{\ddagger} = 57 \pm 2$ kJ mol^{-1} [5]. Comparison of reaction rates in

*Correspondence: Prof. Dr. J. Wirz
Institute of Physical Chemistry
Klingelbergstrasse 80
CH–4056 Basel
Tel.: +41 61 267 38 42
Fax: +41 61 267 38 55
E-Mail: wirz2@ubaclu.unibas.ch

aqueous solution with rates achieved by enzymes reveals the striking efficiency of the latter.

Phototriggers for Time-Resolved Studies of Biochemical Response

Photoactivatable protecting groups can provide temporal and spatial control over the release of active compounds. Such compounds are becoming important tools for the analysis of fast processes in biology and other fields, such as the dynamic flow of liquids or gases. Most phototriggers are based on the *o*-nitrobenzyl photoprotective group.

Current wisdom holds that the release of anions from *o*-nitrobenzyl compounds proceeds by the mechanism shown in *Scheme 1*, but many essential details such as pH-dependence, isotope and substituent effects, the nature of the rate-determining step, the pK_a of the *aci*-nitro and bicyclic intermediates, and the origin of frequently observed nonexponential decay of the *aci*-nitro intermediates are open to question. Buffers are commonly employed to establish pH, and the possible effect of general acid or base catalysis is usually ignored. The properties of protecting groups often do not meet the requirements for optimal performance in biochemical applications. Release rates of ATP nitrobenzyl precursors are on the order of 10 s^{-1} at room temperature, and the side product is toxic. Often, it is not known how fast and efficient the release of the desired biochemicals actually is. There is need for mechanistic studies and room for improvement by the development of faster and more efficient phototriggers [6].

Novel reactions for the use as photoactivatable triggers or for photoaffinity labeling are needed. In the desyl phosphates developed by *R. Givens* (*Scheme 2*), release is fast ($\tau \approx 10$ ns), but both radical and ionic reactions are competing, and the reaction is strongly solvent-dependent. Novel phototriggers for ionic leaving groups X^-, such as that shown in *Scheme 3*, are being developed.

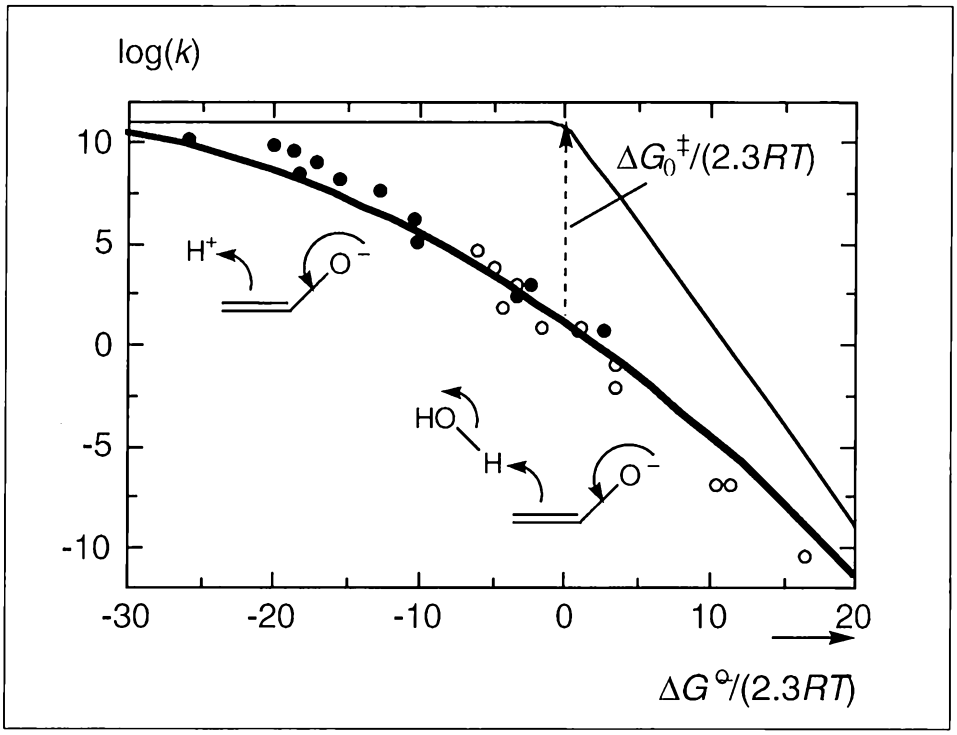
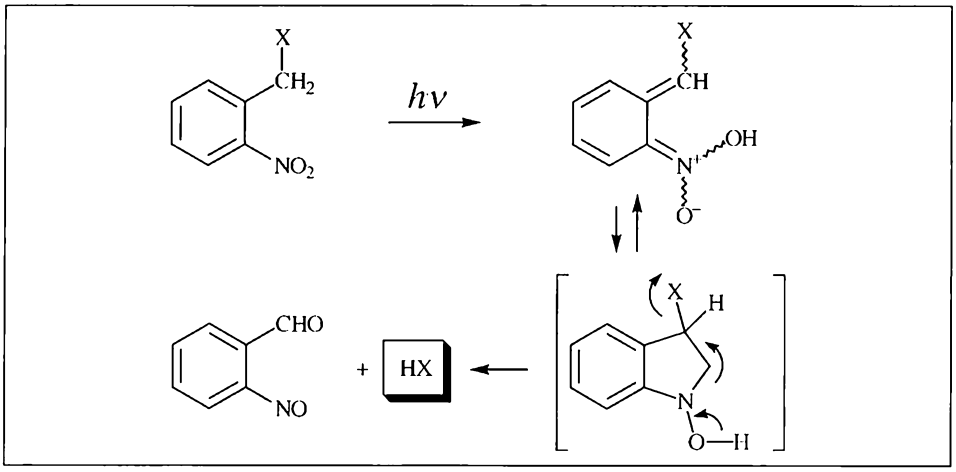
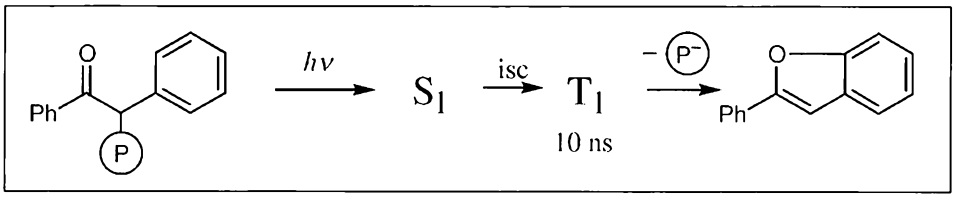


Fig. 1. *Empirical relationship between free energies of reaction and ketonization rate constants of enolates*

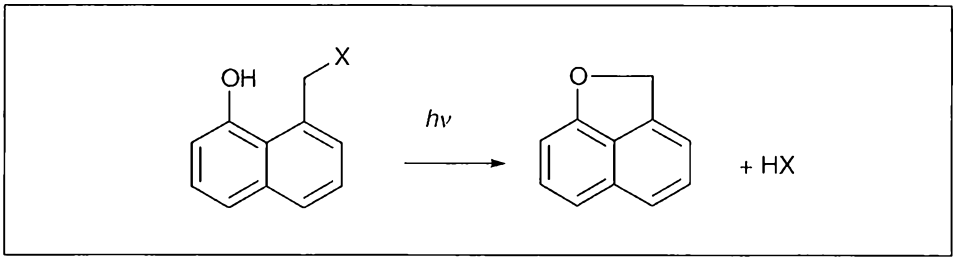
Scheme 1



Scheme 2



Scheme 3



Protein Folding

Understanding how proteins fold is a central problem in biochemistry. Much of the earlier work was limited to the millisecond time scale and above, and the development of methods with much better time resolution is required to investigate folding kinetics of local domains. Meas-

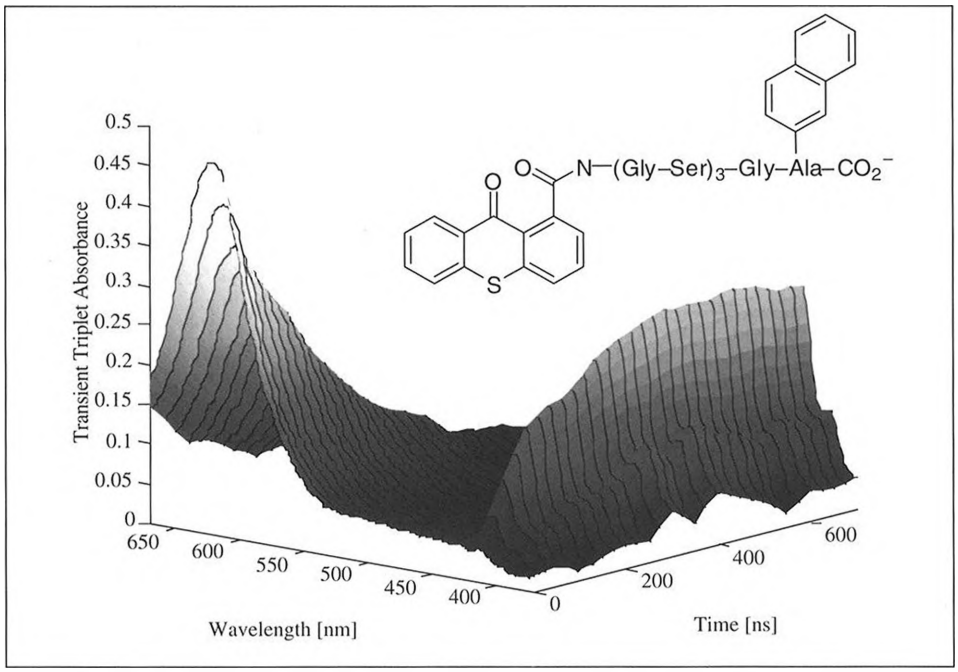


Fig. 2. *Spectrographic tracing of triplet-energy transfer from thioxanthone to naphthalene*

urement of triplet-energy transfer rates between chromophores covalently bound to designed model oligopeptides provides a tool for studying these rates in aqueous solution, because this reaction requires physical contact between the donor and acceptor group. Triplet-energy transfer from thioxanthone to naphthalene can be measured accurately by monitoring the characteristic triplet-triplet absorptions of these chromophores at 590 and 415 nm after selective excitation of the former at 351 nm (*Fig. 2*). In collaboration with *T. Kiefhaber*, we currently measure *intramolecular* rates of triplet-energy transfer from thioxanthonyl to naphthyl attached at varying positions of oligopeptides such as that shown to the left.

Some of the work described above is or was done in collaboration with the research groups of *W. Adam*, Würzburg; *R. Givens*, Lawrence, Kansas; *M. Goeldner*, Strasbourg; *A.J. Kresge*, Toronto; *T. Kiefhaber*, Biozentrum, Basel.

Received: February 26, 1999

[1] W. Adam, C. van Barneveld, O. Emmert, H.M. Harrer, F. Kita, A.S. Kumar, W. Maas, W.M. Nau, S.H.K. Reddy, J. Wirz, *Pure Appl. Chem.* **1997**, *69*, 735.
[2] W. Adam, W.T. Borden, C. Burda, H. Foster, T. Heidenfelder, M. Heubes, D.A. Hrovat, F. Kita, D. Scheutzow, J. Wirz, *J. Am. Chem. Soc.* **1998**, *120*, 593.
[3] R. Born, C. Burda, P. Senn, J. Wirz, *J. Am. Chem. Soc.* **1997**, *119*, 5059.
[4] D.R. McMasters, J. Wirz, G.J. Snyder, *J. Am. Chem. Soc.* **1997**, *119*, 8568.
[5] J. Wirz, *Chemie in unserer Zeit* **1998**, *32*, 311; J. Wirz, *Pure Appl. Chem.* **1999**, *70*, 2221.
[6] L. Peng, F. Nachon, J. Wirz, M. Goeldner, *Angew. Chem.* **1998**, *110*, 3838.