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Investigation of Dynamic Equilibria Using NMR-Spectroscopy
III [1]
Saturation Transfer Experiments on the Hydration of Pyridine-4-Carbaldehyde*

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Spin saturation experiments can be performed by pulse techniques. Different experiments with monoexponential as well as with biexponential decay functions are shown. Such experiments are very useful for the estimation of rate constants of chemical reactions in the time range of 0.1 to 1 seconds, where classical methods as well as relaxation kinetics are unfavourable. The application of spin saturation experiments on the hydration of pyridine-4-carbaldehyde is shown.

(Keywords: Pyridine-4-carbaldehyde; Hoffman-Forsén-method; Spin saturation; Magnetization transfer)

Untersuchung dynamischer Gleichgewichte mittels NMR-Spektroskopie, 3. Mitt.: Magnetisierungstransferexperimente bei der Hydratisierung von Pyridin-3-carbaldehyd

Magnetisierungstransferexperimente können mit Hilfe von Pulstechniken durchgeführt werden. Verschiedene Experimente mit monoexponentiellen als auch mit biexponentiellen Abklingkurven werden gezeigt. Derartige Experimente sind für die Messung der Geschwindigkeit chemischer Reaktionen im Zeitbereich von 0.1 bis 1 Sekunde geeignet, wo die Verfahren der klassischen Kinetik als auch der Relaxationskinetik nicht anwendbar sind. Die Anwendung von Magnetisierungstransferexperimenten auf die Hydratisierung von Pyridin-4-aldehyd wird gezeigt.

Introduction

The investigation of chemical exchange processes by saturation transfer experiments was first performed by *Hoffman* and *Forsén* [2, 3].

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* Dedicated to Prof. Dr. Dr. h.c. *K. Kratzl* on the occasion of his 70th birthday.

Rapid development of the method has made it a powerful tool for the study of kinetic phenomena [1, 4–9]. The use of computer-equipped *Fourier*-Transform spectrometers has further enlarged the field of application and simplified the performance of the experiments.

Spin saturation experiments are in particular useful for the investigation of reversible reaction equilibria with rate constants in the order of seconds where classical kinetic methods as well as relaxation methods fail to succeed in most cases. In the present paper we describe spin saturation experiments on aqueous solutions of pyridine-4-carbaldehyde. Pyridine-4-carbaldehyde (*P4A*) is used as a model compound for the study of elementary reaction steps of pyridoxal phosphate, which is of great importance in biochemistry. Several papers have been published concerning the addition of amines [10–12] and thiols [13] to *P4A*. In aqueous solution these addition reactions are coupled with the hydration of the aldehyde. At neutral *pH* the reaction rate slows down at room temperature to rate constants between $0.05\,s^{-1}$ and $5\,s^{-1}$, convient for the investigation with NMR magnetization transfer techniques.

Experimental

0.1*M* samples were prepared by dissolving 20 µl freshly distilled pyridine-4-carbaldehyde in 2 ml D_2O (to avoid disturbances created by the H_2O-signal).

As *pD* value the reading of a *pH*—meter connected to a glass electrode was taken without further correction. The *pD*—values were kept constant by suitable buffer solutions. NMR-spectra were measured on a Bruker WM 250 spectrometer at a magnetic field of 5.87 T in 5 mm tubes. The deuterium resonance of the solvent provided the field frequency lock. The sample temperature was kept constant by the temperature control unit of the spectrometer.

For the performance of the experiments in the pulse—FT—mode suitable microprograms running on the Aspect 200 computer of the spectrometer have been written.

A basic pulse scheme is shown in Fig. 1; typical aquisition parameters were: number of scans for a given delay: 8; pulse width: 5.9 µs (90°); sweep width: 3 500 Hz; acquisition time 1.1 s; number of delays: 28; relaxation delay: 60 s.

The theoretical basis for all magnetization transfer experiments is given by the *McConnell* equation [4]. The application of this equation on a two spin system yields two linear inhomogeneous differential equations. The coefficients of the general solution of these equations are defined by the boundary conditions of the experiments as given below.

Experiment 1: The time dependence of the signal of nucleus A after sudden saturation of the corresponding signal of B is followed.

Experiment 2: Both signals A and B are saturated; the rf field is removed from A while B remains saturated. The recovery of A is observed.

Experiment 3: The time dependence of A is monitored after the saturation field is removed from B.

Experiment 4: The time dependence of A is monitored after inverting the magnetization at B.

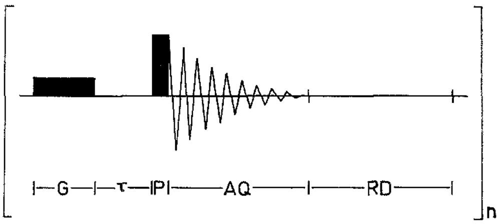


Fig. 1. Basic pulse scheme for saturation transfer experiments. G... Suitable RF—field defining the initial state of the spin system and thus the boundary conditions for the differential equations (saturation, inversion etc.); τ... variable delay; P... π/2—FR—pulse; AQ... Acquisition time; RD... relaxation delay; n... number of scans

Experiment 5: The magnetization of A is inverted and the time dependence of A itself is monitored.

Experiment 6: The signal of A is monitored after removing the saturation field from A.

Experiment 7: A is monitored after removing the saturation field from A and B.

Results and Discussion

In Fig. 2 different pulse experiments (compare Exp. Part) are compared with regard to various parameters: machine time necessary for each experiment, number of exponential functions describing the time

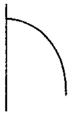
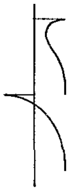
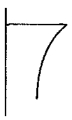
7	4,5	3,6	2	1	EXPERIMENT
					GRAPHICAL REPRESENTATION
SMALL	LARGE	LARGE	SMALL	LARGE	MACHINE TIME
2	2	2	1	1	NUMBER OF EXPONENTIAL FUNCTIONS
1	2	2	2	2	NUMBER OF NECESSARY EXPERIMENTS
MEDIUM	SMALL LARGE	SMALL MEDIUM	SMALL	SMALL	AMPLITUDE

Fig. 2. Comparison of various saturation transfer experiments

dependence of the magnetization; number of experiments which are necessary to obtain all informations for both types of nuclei; amplitude of the exponential function. The machine time needed for each pulse experiment varies from ten hours (large) to about two hours (small) for a typical experiment mentioned in the experimental part—a fact which might be of importance concerning the stability of the NMR-equipment and the sample solution. As one can see from Fig. 2 experiments 2 and 7 are most effective in regard to machine time, especially experiment 7 with which the time dependence of both nuclei can be observed simultaneously (third column). The amplitude of the exponential functions is described by the complete change of magnetization during the *Hoffman-Forsén* experiment. If the amplitude of the exponential function is large like in experiment 5 a favourable signal to noise ratio increases the accuracy of the estimated chemical and magnetic parameters. The small amplitudes of experiment 1 and 2 depend on the behaviour of the chemical system, on the chemical relaxation and the equilibrium constant. Fig. 3 shows the amplitude depending on the ratio of the chemical and the magnetic relaxation at an equilibrium constant $K = 1$. Experiment 1 shows a favourable amplitude for high rate constants whereas experiment 2 has large amplitudes in ranges of slower chemical relaxation. Each experiment therefore is an excellent completion of the other. Except experiment 1 and 2 all other experiments show a biexponential time dependence of the magnetization. Two different methods have been applied for the evaluation of such biexponential functions: 1. The method of *Provencher* [15–

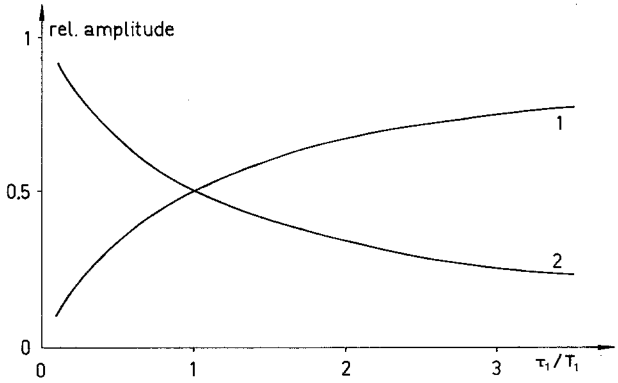


Fig. 3. Relative amplitude of spin saturation experiments 1 and 2 depending on the ratio of chemical and magnetical relaxation time

17], based on a *Fourier* transformation procedure, checking how many exponential terms are included in the set of experimental points. Such methods give good results in the calculation of multiexponential curves; only with data bad signal to noise ratios the procedure sometimes fails in finding a significant parameter set. 2. Special least square fitting procedures [21, 22] also give satisfactory results in most cases. Table 1 shows a comparison of the time constants of the exponential terms estimated from different experiments with the *Fourier* transform procedure as well as from the least square method. A good agreement within the limits of the methods is observed except for experiment 7 where the difference of the amplitude coefficients of both exponential terms is too large. Nevertheless it can be seen that the biexponential functions give the physical parameters with lower accuracy than the monoexponential experiments 1 and 2.

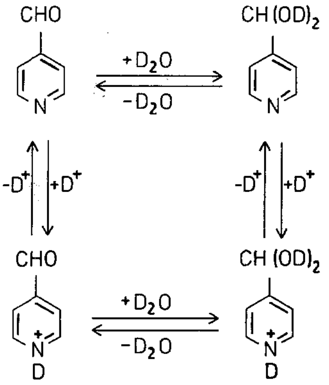
Table 1. *Time constants of the exponential terms according to different spin saturation experiments at* $pD = 6.2$; *unit:* s^{-1}; *experiments 1 and 2 monoexponential, experiments 3 to 7 biexponential*

Experiment	1	2	3	4	5	6	7
method 1	0.155	0.143	0.27	0.29	0.26	0.39	0.099
error	4.5%	3.8%	14%	13%	18%	16%	48%
method 2			0.27	0.29	0.25	0.31	—

The hydration of pyridine-4-carbaldehyde is an excellent system to be measured by spin saturation techniques—an equilibrium constant near to 1 allows the observation of both species. In neutral medium the rate constant of the addition of water is in the time range which is too slow for relaxation experiments and too fast for classical chemical kinetics. Fig. 4 shows the NMR spectrum of *P4A* in aqueous solution at 295 K. The signal of the aldehyde proton and the proton of the hydrated aldehyde are well separated and show no line broadening. In Fig. 5 the *pD* dependence of the logarithm of the reciprocal chemical relaxation time is shown. In acidic as well as in basic solution the hydration steps are accelerated. A minimum of the relaxation time is observed between $pD = 5$ and $pD = 6$. The hydration behaviour of *P4A* is described by reaction scheme 1.

In Scheme 1 two protonation steps are coupled with two hydration steps. The thermodynamic constants in aqueous solution have been analyzed and the corresponding data are given elsewhere [21–25]. The dynamics of the reaction system have been investigated earlier by calorimetric and stopped flow methods [23–26], where small amounts of aldehyde have been mixed rapidly with large amounts of water. The resulting chemical relaxation time can be estimated only with relative low accuracy. Additional the dynamic reaction system is more complex because the hydration steps follow acid-base catalysis [27, 28]. The relaxation time for one hydration step can be expressed by the following equation:

Scheme 1



$$\tau^{-1} = k_{D^+} \cdot c_{D^+} + k_{OD^-} \cdot c_{OD^-} + k_{H_2O}$$

where k_{H^+} describes the acid catalysed reaction step; k_{OH^-} the base catalyzed one and k_{H_2O} describes the catalysis by the solvent. If the catalysis by the solvent is slow, a plot of log (1/τ) versus *pD* should yield a straight line of slope − 1 or + 1 depending on whether the reaction is acid or base catalyzed. The proton transfer reactions of the protonation and the deprotonation of the pyridine nitrogen occur much faster than the hydration steps. In such cases, if the rates of two reaction steps are different by more than a factor 10, the faster reaction is not influenced at all by the slow one, and the slow reaction step is only influenced by the equilibrium of the fast process. In Fig. 5 the range from $pD = 6$ to higher *pD* values is mainly described by the base catalysis of the hydration of *P4A*. Below $pD = 3.5$ the hydration of the protonated *P4A* is observed and follows acid catalysis. Between $pD = 3.5$ and $pD = 5$ coupling between the hydration of the protonated and the deprotonated species is observed.

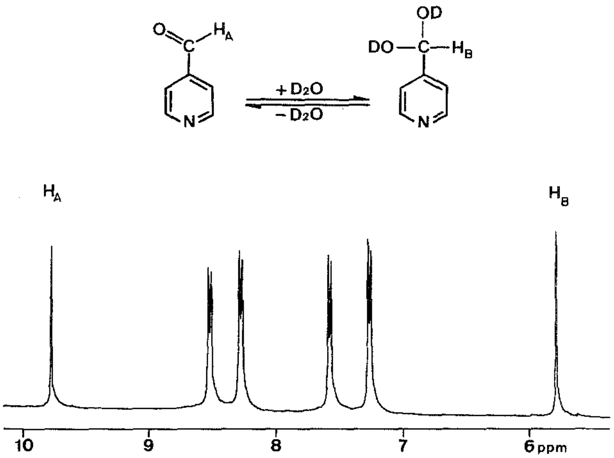


Fig. 4. ^{1}H-NMR-spectrum of *P4A* in D_2O, $T = 295$ K

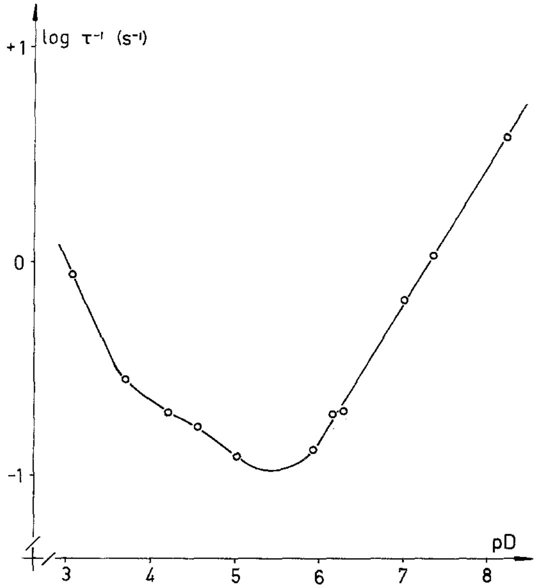


Fig. 5. Logarithm of the reciprocal chemical relaxation time depending on the *pD*-value

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