Tetrahedron Vol. 44, No. 9, pp. 2483 to 2492, 1988
Printed in Great Britain.

0040-4020/88 $3.00+.00

BASE CATALYZED PROTON EXCHANGE RATES OF A MODEL CYCLOPEPTIDE IN WATER-DIMETHYLSULPHOXIDE SOLUTIONS

J. CHRISMENT, J.-J. DELPUECH* and W. RAJERISON*

Laboratoire d'Etude des Solutions Organiques et Colloïdales, UA CNRS 406, Université de Nancy I, B.P. 239, 54506 Vandoeuvre-les-Nancy Cedex, France

(*Received in Belgium* 24 *February* 1988)

Abstract - Proton tranfers on the peptide bond of *cyclo*-Glycyl-L-Prolyl (PH) were studied at 25°C by DNMR in seven H_2O/DMSO mixtures (SH) ranging from pure water to anhydrous DMSO. Two kinetic processes (I) and (II) are observed, catalyzed by the conjugate base of either the solvent (OH^-) or of the peptide (P^-). Mechanisms (I) and (II) were observed alone in the most or least aqueous solvents, respectively. The rate constant of mechanism I, $k_I \sim 10^9\ M^{-1}s^{-1}$ is nearly constant along the series of solvents, being levelled to its diffusion limit. The backward reaction is made slower by addition of DMSO, so that the pK of the peptide (pK_{NH}) becomes smaller than that of the solvent (pK_{SH}) in the least aqueous mixtures, pK_{NH}=18.3 against pK_{SH}=22 to 33. Rate constants for mechanism (II) increase from pure DMSO ($1.81\times10^6 M^{-1}s^{-1}$) to the more aqueous solvents ($\sim5\times10^8 M^{-1}s^{-1}$ when x_{DMSO}=0.35), mainly because of bridging water molecules transferring protons within the acid-base pair PH/P^- through a Grotthus mechanism.

Résumé - Les transferts protoniques sur la liaison peptidique de la *cyclo*-Glycyl-L-Proline (PH) sont étudiés par RMN dynamique dans sept mélanges H_2O/DMSO allant de l'eau pure au DMSO anhydre, à 25°C. Deux processus cinétiques (I) et (II) sont observés, catalysés par la base conjuguée soit du solvant (OH^-) soit du peptide (P^-). Le mécanisme (I) fut seul observé dans les solvants les plus aqueux, et de même le mécanisme (II) dans les milieux les moins aqueux. La constante de vitesse du premier, $K_I \sim 10^9 M^{-1}s^{-1}$, est pratiquement constante sur toute la gamme de solvants, par nivellement à la limite imposée par la diffusion. La réaction inverse est ralentie par addition de DMSO, si bien que le pK du peptide (pK_{NH}) devient plus petit que celui du solvant (pK_{SH}) dans les milieux les moins aqueux, pK_{NH}=18.3 contre pK_{SH}=22 à 33. La constante de vitesse du mécanisme(II) augmente depuis le DMSO pur ($1.81\times10^6 M^{-1}s^{-1}$) jusqu'aux solvants plus aqueux ($\sim5\times10^8 M^{-1}s^{-1}$ pour x_{DMSO}=0.35), principalement en raison de molécules d'eau intermédiaires transférant les protons à l'intérieur du couple acido-basique PH/P^- selon un mécanisme de Grotthus.

INTRODUCTION

Previous investigations on the acid-base properties of nitrogen atoms in amides[1] and peptides[2] have shown that proton abstraction in basic aqueous or non-aqueous solutions of these substrates (PH) takes place by either of these two kinetic processes, involving the conjugate base of either the solvent (S^-) or the substate (P^-) according to the equations:

$$PH + S^- \xrightarrow{k_I} P^- + SH \qquad (I)$$

$$PH + P^- \xrightarrow{k_{II}} P^- + PH \qquad (II)$$

The first process was found to be fully predominant in H_2O solutions of a model amide, N-methylacetamide (NMA)[1], while the second process alone is observed in DMSO solutions of NMA [1] or of a doubly protected dipeptide Gly-Gly (PG_2) or tripeptide Gly-Gly-Gly (PG_3)[2]. However the above peptides are not soluble in water where, in any case, they would be very sensitive to the hydrolysis of their protecting ends.

The present investigation uses a self-protected cyclopeptide *c*-Gly-L-Pro (cyclo-Glycyl-L-Prolyl) which is soluble both in pure water and in anhydrous DMSO without suffering hydrolysis. The expected switch from mechanism (I) to mechanism (II) was explored by using a series of DMSO/H_2O mixtures, covering the whole range from pure water to DMSO. To each of these mixtures (SH) were added small amounts of base and the level of acidity was measured with Hammett indicators. Rate constants k_I and k_{II} were determined from the NMR lifetimes τ_{PH} of the amide hydrogen as a function of the pH and the analytical concentration C ~ [PH] of the peptide according to eqn 1:

(*) Present adress: Laboratoire de Chimie Organique (Pr. Rabesiaka), Université de Tananarive, Madagascar.

$$1/\tau_{PH} = k_I[S^-] + k_{II}[P^-] \quad (1)$$

or

$$1/\tau_{PH} = (k_I K_{SH} + k_{II} K_{NH} C)/[H^+] \quad (2)$$

K_{NH} is the ionization constant of the peptide and K_{SH} is the apparent autoprotolysis constant for the solvent mixture[3-4]:

$$K_{SH} = [S^-][H^+]$$

with $[S^-] = [DMSO^-] + [OH^-]$

and $[H^+] = [H_3O^+] + [DMSO...H^+]$

K_{SH} values for the mixtures investigated were taken from the work of Schaal *et al.*[5-8]. In fact, in the pH range explored, the concentration of the dimsyl anion is negligible with respect to that of the hydroxide ion, except in anhydrous DMSO, so that mechanism (I) can be actually written as:

$$PH + OH^- \xrightarrow{k_I} P^- + H_2O \quad (I)$$

The investigations included measurements of the pK of the peptide in these solvents, an important aspect of this work since acid-base properties of nitrogen atoms in amides and peptides are still poorly documented.

EXPERIMENTAL SECTION

Materials and solutions

The cyclopeptide is a Bachem product used without further purification. DMSO is purified according to procedures described previously[9]. Bidistilled water (conductivity of *ca.* $10^{-6}ohm^{-1}.cm^{-1}$) was used to prepare aqueous solutions of DMSO. Among the series of H_2O/DMSO mixtures studied by Schaal *et al.*[5-8], we selected seven solvent mixtures containing respectively a weight percentage of DMSO of 0 (pure H_2O), 61.2 (A), 70.5 (B), 80.4 (C), 91.0 (D), 95.5 (E) and 100 (pure DMSO). Basic solutions used to promote proton transfers were obtained by small additions (~10^{-4} to 10^{-3} M) of either 97-99% triethylamine (EGA Chemie) to pure water, or tetramethylguanidine (FLUKA, puriss. grade) to pure DMSO, or tetraethylammonium hydroxide (FLUKA, 40% in water) to H_2O/DMSO mixtures A to E.

pH measurements

For each solution, two pH ranges are explored to determine either the pK of the peptide or the pH around coalescence. In each case, the standard state used to define the activity coefficients is the infinitely diluted solution of the solute in the H_2O/DMSO mixture under consideration. Activity coefficients of neutral species were taken equal to unity, this assumption is probably true in the concentrations used here (a piece of evidence suggesting the validity of these assumptions is the independence of the ratio $[I^-]/[IH]$ of the ionized to the unionized forms of Hammett indicators in the concentration range of *ca.* 10^{-4}-10^{-5}M). Moreover if the Debye-Hückel law holds in DMSO solutions, then the activity coefficients of ionic species assumed to be of a similar size are equal in a given solution to a common value $\gamma_\pm$, and therefore cancel in the computation of species $[P^-]$ and $[S^-]$ from the ratio $[I^-]/[IH]$:

$$\frac{[P^-]}{[PH]} \Big/ \frac{[I^-]}{[IH]} = K_{NH}/K_{IH}$$

$$[S^-] \Big/ \frac{[I^-]}{[IH]} = K_{SH}/K_{IH}$$

even if the term $\gamma_\pm^2$ (itself very close to unity) appears in the formulation of the ionization constants:

$$K_{IH} = \gamma_\pm^2 \, [H^+][I^-]/[IH]$$

$$K_{SH} = \gamma_\pm^2 \, [S^-][H^+]$$

$$K_{NH} = \gamma_\pm^2 \, [P^-][H^+]/[PH]$$

In pure water, the pH's at coalescence were determined using a combined glass electrode METROHM EA 159 (the pK of the cyclopeptide was not accessible due to the levelling effect of water).

In pure DMSO, pH measurements were carried out using an absolute pK scale described previously[1]. The Hammett indicators[10] required for the present investigations are (a) 4-nitroaniline, pK=19.2, used to determine the pH of a 0.02M solution of the cyclopeptide titrated with hexamethyldisilazane (Fluka) under argon in a glove-box (b) 2-bromo-4,6-dinitroaniline, pK=13.4, used in the coalescence range.

In H_2O/DMSO mixtures, absolute pK scales have been settled by Schaal *et al.*[5-8] from a combined potentiometry-spectrophotometry method.The pK of indicators (pK_{IH}), as well as the apparent pK of the solvent (pK_{SH}, see above), were read from plots of these quantities as a function of the weight percentage of DMSO. The Hammett indicators used to measure the pK of the cyclopeptide (from the pH at half-neutralization with tetramethylammonium hydroxide) are: 4-nitrodiphenylamine in solvent C (pK_{IH}=16.6) and 2-nitrodiphenylamine in solvents D and E (pK_{IH}=18.5 and 18.7). No pK measurement could be done safely for the most aqueous mixtures A et B because of the levelling effect of water, i.e. of the too small pK difference between the solvent (pK_{SH}=16.65 and 17.78, respectively) and the peptide (pK_{NH} ~ 14-16, see below). The pK_{IH} values for solutions A to D were checked according to an absolute method[1] using an excess of the strong base S^- (OH^-) and measuring the equilibrium:

$$IH + S^- \overset{K_e}{=} I^- + SH$$

K_{IH} is then deduced from the knowledge of K_e (this work) and K_{SH} (ref.5) according to the equation

$$K_{IH} = K_e K_{SH}$$

K_e is obtained by measuring the apparent extinction coefficient $\epsilon' = d/a$ (d: optical density ; a: analytical concentration of the indicator) as a function of the concentration of added base $[S^-]$, and using the relationship

$$1/\epsilon' = 1/\epsilon + (1/\epsilon K_e)/[S^-]$$

where ϵ is the extinction coefficient of the ionized form of the indicator $[I^-]$.

For a constant concentration of the indicator, a good linearity of $1/\epsilon'$ *vs* $1/[S^-]$ is observed. The least squares slope yields ϵK_e, and the intercept ϵ itself, from which K_e and ϵ are deduced. For each couple indicator/solvent, three straight lines are drawn, corresponding to three different concentrations a of the indicator (Table 1).

(1) *Solvent C (pK_{SH} = 19.32) and 4-nitrodiphenylamine (λ = 505 nm).*

10^2x[OH$^-$](M)	10^5xa(M)=4.20	3.15	2.1
0.055	1.47	1.11	0.74
0.110	1.68	1.28	0.86
0.165	1.75	1.33	0.90

K_e = 5432

(2) *Solvent D (pK_{SH} = 22.06) and 2-nitrodiphenylamine (λ = 545 nm).*

10^2x[OH$^-$](M)	10^5xa(M)=1.59	1.19	0.795
0.125	1.10	0.83	0.55
0.250	1.22	0.92	0.61
0.500	1.29	1.96	0.64

K_e = 3432

Table 1. Spectrophotometric measurements of the absorbance (d) of Hammett indicators (IH) of analytical concentration a in H_2O/DMSO mixtures C and D as a function of added base OH^- at 25°C. Constants K_e for the equilibrium $IH+OH^- = I^- + H_2O$ are shown below each series of experiments.

The agreement with the values from the literature[6] is very good, in spite of a 5°C difference of temperature in the two series of measurements: pK_{IH} = 15.6 (this work) against 15.6(C) ; 18.5 and 18.5(D). The method is unfortunately valid only when the concentration of the added base necessary to obtain pH ~ pK_{IH} is not too small ($[S^-] >\sim 10^{-3}$M). Otherwise acidic or basic impurities from the solvent made the results fully erratic, even if a correction is brought in the calculation of $[S^-]$ when the concentration of the base is of the same order of magnitude as that of the indicator. The method is thus restricted to pK's ranging from pK_{SH} to *ca.* pK_{SH}-3, excluding the less aqueous mixture (E) where pK_{SH} = 24.0 while pK_{IH} = 18.7. Although restricted to the more aqueous mixtures, these measurements show a very good agreement with those of Schaal *et al.*[6] and thus allow us to use their value for solution E with a high degree of confidence.

A similar situation prevails for the Hammett indicators used to determine the pH of solvents D and E at coalescence, respectively the 2,4-dinitrodiphenylamine (pK = 12.7), and 4-nitrodiphenylamine (pK = 16.5)[6]. For solutions A to C, there is no direct measurement of the pK of 4-nitrophenol, used in the present work as an indicator of pH at coalescence. The pK values quoted in the literature[7] result from ρ-σ Hammett correlations using spectroscopic data. The absolute method described above cannot be used here due to the much more acidic nature of the indicator (pK_{IH} ~ 10). To circumvent this difficulty, there exists a general method due to Hammett using a series of overlapping indicators. We found such a series going from 2,4-dinitrodiphenylamine (pK_{IH} = 12.9) to 4-nitrophenol, in solution B only, by using thymol blue as an intermediate indicator. In a first step, the auxiliary pK of thymol blue in B was determined by adding to B 2.9×10^{-6} and 1.6×10^{-5}M of the first two indicators of the series. The $[I^-]/[IH]$ ratios for both indicators were determined by spectrophotometry (at λ_{max} = 500 and 610 nm, respectively), thus yielding the Δ pK between the two indicators, and therefore the pK of thymol blue itself (pK = 11.3). The same operation was repeated in a second step using again thymol blue and 2.8×10^{-5}M 4-nitrophenol (λ_{max} = 417 nm), leading to pK = 10.3. This value is clearly higher than the one quoted above from spectroscopic data (pK = 8.6 after a correction[12] from 20° to 25°C), but is in good agreement (pK = 10.28) with potentiometric measurements in a H_2O/DMSO mixture whose composition is quite close to that of solution B[13]. The above discrepancy may be due to an exaltation of mesomeric effects in the nitrophenolate ion (vitiating the σ-substituent parameter taken as a constant in the various H_2O/DMSO mixtures), and/or to specific solvation effects bringing forth unexpected deviations in spectroscopic data. For solutions A and C, the above method of overlapping indicators did not work because of too large ΔpK values. We used therefore the pK's derived from spectroscopic correlations mentioned above[12], which are probably underestimated values: pK = 8.3 and 9.0, respectively. The interpretation of our kinetic results suggests somewhat higher values: pK = 9.3 and 9.5 (see below).

All spectroscopic measurements were carried out at 25°C using a UV-visible spectrophotometer VARIAN DMS 100 and 1cm HELLMA quartz cells.

NMR spectroscopy

Proton spectra were recorded on a BRUKER AM 400 spectrometer at 400 MHz and 25°C. Kinetic data were obtained as described in a previous publication[1]. The coalescence of the N-methylene lines (born by carbon C(α), see formula in figure 3) as a function of the pH was used to obtain rate constants for the exchange of the peptide hydrogen according to eqn.2.

SOLVENT	δ_X(NH)	δ_A and δ_M(CH_2)		$^2J_{AM(HH)}$	$^3J_{AX}$
H_2O	8.1125	3.8858	4.1768	-17.32	4.48
A (61.2 % DMSO)	8.1623	3.7342	4.1006	-16.60	4.52
B (70.5 % DMSO)	8.0874	3.6174	4.0049	-16.94	4.22
C (80.4 % DMSO)	8.1376	3.6312	(*)	-16.68	4.28
D (91.0 % DMSO)	8.1073	3.5621	4.0136	-16.52	4.54
E (95.5 % DMSO)	8.0875	(*)	4.0050	-16.52	(*)
DMSO	8.0892	3.5045	3.9913	-16.48	4.52

(*) Lines overlapping with H_2O resonance.

Table 2. Chemical shifts δ(ppm/TMS) and coupling constants J(Hz) for the AMX spectrum of the Glycyl residue of *c*-Gly-L-Pro in H_2O/DMSO mixtures, at 400 MHz and 25°C.

The NH lines themselves were not sufficiently sharp for this purpose due to quadrupolar relaxation of the coupled nitrogen nucleus. The static spectrum consists, besides the lines of DMSO and water, of a broadened singlet for the peptide hydrogen at *ca* 8 ppm (table 2), a set of lines between 3.5 and 4.1 ppm for the $CH_2(\alpha)$ protons (Fig 1a), four sets of lines around 4.2, 2.2, 1.8 and 3.4 ppm for protons born by carbons α' β' γ' δ of the proline ring.

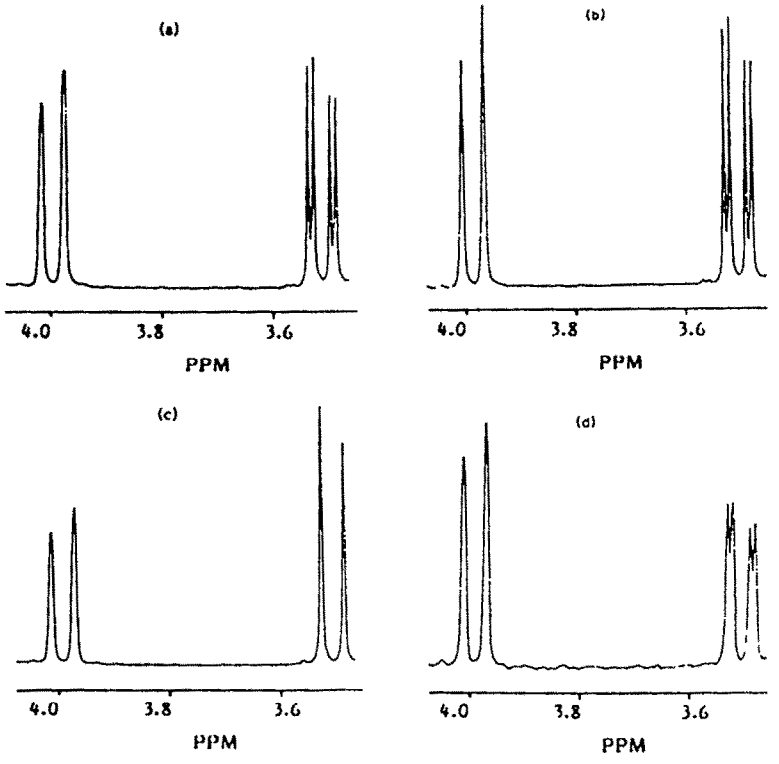

Fig. 1. ^1H NMR spectra ($CH_2(\alpha)$ protons only) at 400 MHz and 25°C of 0.1 M DMSO solutions of cyclo-Glycyl-L-Prolyl. (a) undecoupled static spectrum (b) static spectrum with $CH(\alpha')$ decoupling, (c) or with N-H(x) decoupling (d) undecoupled spectrum in DMSO containing $2.9.10^{-3}$M tetramethylguanidine ($\pi\nu_{1/2}$= 2.7 s^{-1}).

The glycyl methylenic lines (3.5 to 4.1 ppm) were analyzed as usually[14-15] on the basis of the AB part of an ABX pattern, which reduced here to the AM part of an AMX pattern due to the high working frequency. The spectrum then consists of two quadruplets at high and low fields for the non equivalent methylenic protons, say A and M, respectively. An interesting peculiarity is the presence of a broadened doublet only in the low-field part of the spectrum (Fig 1a). In fact, each component of the doublet is itself an ill-resolved doublet with a small line separation of 0.6 Hz. However, this splitting is not due, as expected, to a small coupling J_{MX} between peptide NH(X) and methylene H(M) protons.

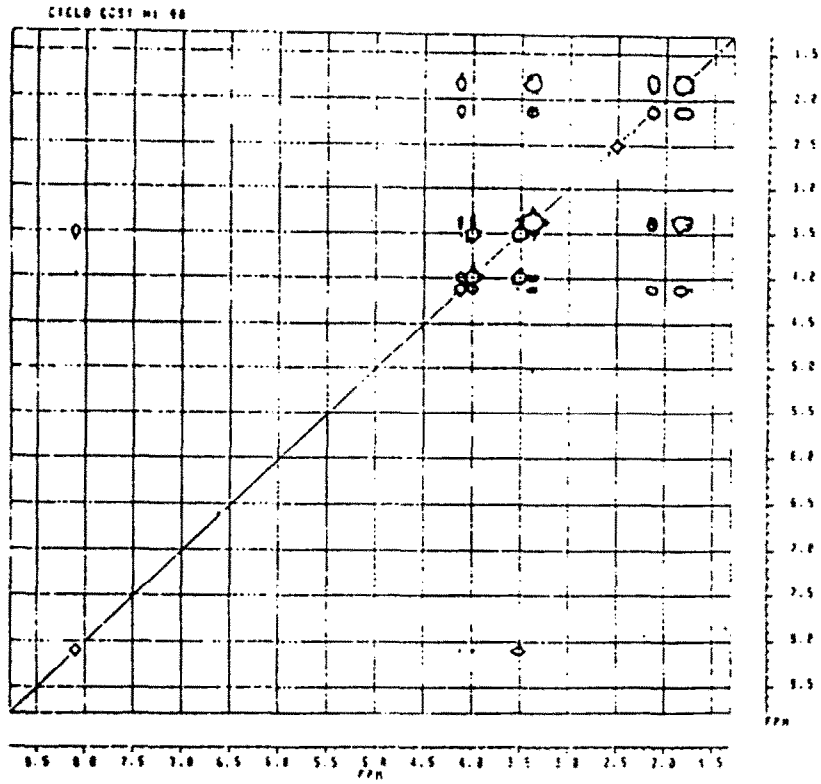

Fig. 2. ^1H COSY 2D-NMR spectrum at 400 MHz and 25°C of a 0.1 M DMSO-d_6 solution of cyclo-Glycyl-L-Prolyl (16 scans over 1Kx1K points, digital resolution: 2.92 Hz ; contour plot ; lines subscripted as shown in the text).

This is clearly shown on a 2D COSY proton spectrum[16] (Fig 2) where correlation lines can be drawn between protons H(A) and H(X), H(A) and H(M) (as expected), and H(M) and CH(α'). We must therefore conclude that $J_{MX} \approx 0$ and that the small splitting of the M doublet is due to a long-range coupling between the α-protons of the glycyl and prolyl residues. This is confirmed by usual 1D experiments in which either the CH(α') lines or the NH(X) lines are selectively irradiated (Figs 1b and 1c). This reveals a 90° dihedral angle φ between planes N-C(α)-H(M) and H-N-C(α) according to Karplus law.

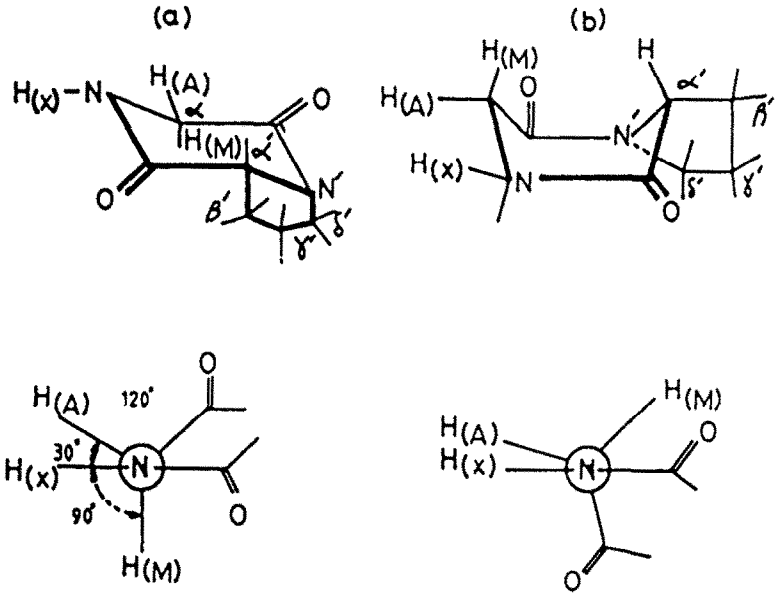

Fig. 3. Perspective and Newman (along the C(α)-N bond) projections of the chair-like (a) and boat-like (b) conformations of cyclo-Glycyl-L-Prolyl.

Two conformations may be imagined for the cyclopeptide ring (Fig 3) either a boat-like conformation, in which the two peptide bonds are planar, or a distorted chair-like conformation in which there is a torsion angle ω of about 60° in each peptide bond. Molecular models then show that only the second conformation can lead to an α angle of 90° between the approximately equatorial NH(X) and axial H(M)(α) protons (in fact α is not equal to 60° as in cyclohexane due to distorsions introduced by nitrogen atoms and carbonyl group). Proton H(A) appearing at high field is therefore assigned to the pseudo-equatorial position (contrary to what is observed in cyclohexane or piperidine derivatives) with an expected value J_{AX} of about 4.5 Hz due to a dihedral angle of 30° (see the Newman projection in figure 3).

Coupling constants J_{AX} and J_{MX} are not sensitive to the solvent composition (Table 2), this shows no variation in the cyclopeptide conformation induced by the solvent. The chemical shift δ_x of the peptide hydrogen was found almost constant (at ± 0.03 ppm) while, more surprisingly, the α-methylenic protons were solvent dependent, the difference $(\delta_A - \delta_M)$ going from 0.49 ppm in DMSO to 0.29 ppm in H_2O.

NMR rate constants $1/\tau_m$ were obtained from the coalescence of either A doublet, or both (Fig 1d). This was however impossible for the less aqueous solvent mixture E, because of an overlap of the A doublets with the line of water moving downfield when the content of DMSO is increased. In this case, we used the linewidth of the water line itself $\Delta\nu_{1/2}$, yielding the mean lifetime of water protons[16]:

$$\tau_{H_2O} = 1/\pi\Delta\nu_{1/2} \qquad (3)$$

This allows us in turn to deduce the part of the exchange on the peptide hydrogen $1/\tau'_{PH}$ ($\leq 1/\tau_{PH}$ involving a water molecule according to the formula[17]:

$$1/\tau'_{PH} = 1/\tau_{H_2O} \cdot \frac{2[H_2O]}{[PH]}$$

This exchange concerns in principle only mechanism (I) (see however the discussion below), i.e. $1/\tau'_{PH}$ should be equal to $k_I[OH^-]$. The interpretation of the results thus obtained for solution E required further a test experiment in another solvent (D) where both types of coalescences (α-methylenic and H_2O lines) were observed and analyzed.

RESULTS AND DISCUSSION

Two or three sets of kinetics experiments were performed for each solvent using two or three concentrations of peptide, [PH] = 0.05; 0.1; 0.15 or 0.2, and up to five pH values (Table 3). Plots of $1/\tau_{PH}$ versus $1/[H^+]$ are straight lines of slope p with a zero intercept and least squares correlation coefficients larger than 0.99.

Measurements in pure water

Slopes p were found independent from the peptide concentration (Table 3).

SOLVENT	[PH](M)=0.05 τ'^{-1}_{PH}	*pH*	0.10 τ'^{-1}_{PH}	*pH*	0.15 τ'^{-1}_{PH}	*pH*	0.20 τ'^{-1}_{PH}	*pH*
H_2O	0.5	4.6	0.5	4.6				
	1.4	5.0	1.4	5.0				
	2.7	5.3	2.7	5.3				
	4.3	5.5	4.3	5.5				
A	1.2	8.34			1.9	8.35		
	1.8	8.41	2.0	8.46				
	3.2	8.61	3.2	8.66				
			5.0	8.65	5.2	8.90		
B	0.6	9.04						
	1.1	9.31	1.2	9.15	1.6	9.02		
	2.1	9.61	2.5	9.40	2.7	9.29		
	3.4	9.80	4.5	9.67	4.7	9.53		
			6.0	9.80				
C	0.7	9.80						
	1.7	10.18	1.5	9.90	1.7	9.80		
	2.0	10.23	2.4	10.10				
			3.5	10.27	2.8	10.00		
					3.2	10.08		
D	0.6	11.90						
	1.3	12.20						
	2.0	12.40	0.7	11.75				
			1.4	12.00	1.6	11.9		
			1.9	12.15				
					3.0	12.15		
DMSO	1.5	13.60	1.0	13.00			1.0	12.70
	2.3	13.75	1.9	13.30			1.7	13.00
	4.2	14.00	4.2	13.60				
	5.5	14.15	5.4	13.75			4.5	13.40

Table 3. NMR rate constants τ'^{-1}_{PH}, in s^{-1}, as a function of the *pH* in DMSO/H_2O mixtures containing various amounts of peptide PH, at 25°C.

Mechanism I alone is then assumed to occur in pure water and the unique p value thus yielded the overall rate constant $k_1=k_IK_w$ (from eqn 2, with $K_{SH}=K_w$), and therefore $k_I(H_2O)=1.36\times10^{9}s^{-1}$ (Table 4). This value is close to the diffusion-limited rate constant $k_d\sim10^{10}s^{-1}$ in water and $5\times10^{9}s^{-1}$ in DMSO[1]. This agrees fairly well with the prediction we made in a previous paper[2], considering the k_I value obtained for N-methylacetamide (NMA), $k_I(H_2O)=1.56\times10^{6}s^{-1}$, a chemically analogous compound, but much less acidic than the peptide presently investigated (by five orders of magnitude in DMSO: $pK_{NH}=23.4$[1] against 18.3).

SOLVENT	H_2O	A	B	C	D	E	DMSO
x_{DMSO}	0.000	0.266	0.355	0.486	0.700	0.830	1.000
$[H_2O]$ (M)	55.5	23.2	17.8	11.8	5.55	2.77	<0.01
pK_{SH}[a]	14.0	16.65	17.78	19.32	22.06	24.0	33.0
pK_{NH}	—	—	—	16.6	18.3	18.25	18.3
k_1 ($M.s^{-1}$)	1.36×10^{-5}	7.01×10^{-9} 6.92×10^{-8}[b]	5.52×10^{-9}	3.54×10^{-11} 1.10×10^{-10}[b]	1.17×10^{-13}	—	—
k_I ($M^{-1}.s^{-1}$)	1.36×10^{9}	3.13×10^{8} 3.09×10^{9}[b]	3.33×10^{9}	7.39×10^{8} 2.29×10^{9}[b]	1.34×10^{9}	—	—
k_{-1} ($M^{-1}.s^{-1}$)	—	—	—	1.38×10^{6} 4.36×10^{6}[b]	2.29×10^{5}	—	—
k_2 ($M.s^{-1}$)	—	—	4.22×10^{-7}	1.57×10^{-9} 4.87×10^{-9}[b]	1.30×10^{-11}	2.01×10^{-12}	9.07×10^{-13}
k_{II} ($M^{-1}.s^{-1}$)	—	—	—	6.24×10^{7} 1.94×10^{8}[b]	2.59×10^{7}	3.58×10^{6}	1.81×10^{6}

(a) From reference 5
(b) Corrected values (see the text)

Table 4. Kinetic (k_1, k_2, k_I, k_{-I}, k_{II}) and thermodynamic (pK_{SH}, pK_{NH}) data for peptide hydrogen abstraction of cyclo-Gly-L-Pro in basic DMSO/H_2O solvent mixtures of given composition (x_{DMSO} or $[H_2O]$), at 25°C.

Measurements in pure DMSO

Slopes p were found strictly proportional to the peptide concentration C, with a coefficient $k_2=k_{II}K_{NH}$. As in the case of NMA[1], PG_2 and PG_3[2], these observations are consistent with proton abstraction by the conjugate base of the peptide according to mechanism (II). In this case, mechanism (I) has a negligible contribution to the proton transfer, since k_1 is at most equal to *ca.* 10^{-24} ($K_{SH}=10^{-33}$ and $k_I \leq k_d\sim10^{9}$-10^{10}), while $k_2=9.07\times10^{-13}Ms^{-1}$ (Table 5). Compared to those relative to NMA and PG_2, rate constants k_{II}(DMSO) follow an order of increasing magnitude:

NMA(2.13×10^{9})>>PG_2(3.6×10^{6})>*c*-Gly-Pro(1.81×10^{6})

This order reflects the sequence of decreasing pK's for the amide or peptide bond, $pK_{NH}=23.4$; 19.4 and 18.3 respectively. This parallelism is coherent, but not necessary, since an increased acidity of the NH bond in PH is accompanied by a decreased basicity, and presumably nucleophilicity, of the conjugate base P^-, thus making predictions over the rate constant k_{II} uncertain.

Measurements in solvent mixtures A to D

The contribution of mechanism (II) was found negligible in solvent A ($k_1 >> k_2C$). In mixtures B,C,D, k_1 and k_2 values could be determined from the plots of p *versus* C. From experimental k_1 values, we deduced rate constants k_I from the knowledge of K_{SH} (Table 4). Rate constants k_{II} can be computed for those solvents (C and D)

in which the ionization constant K_{NH} can be measured. Rate constants k_I and k_{II} are displayed on a semi-logarithmic plot as a function of the solvent composition x_{DMSO} (Fig 4).

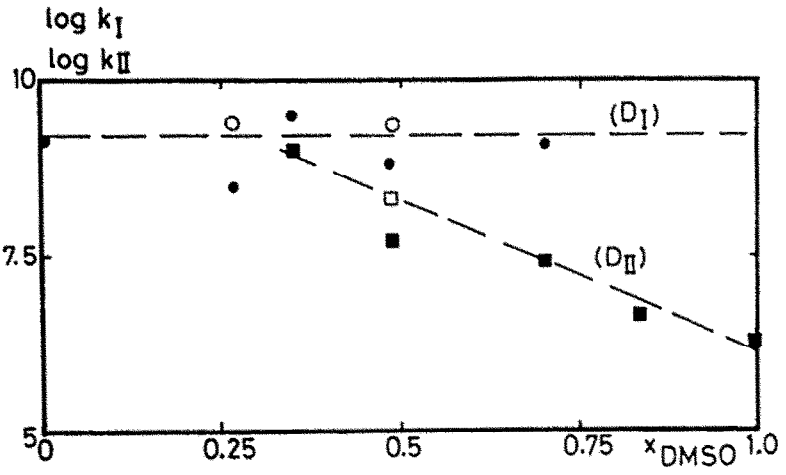

Fig. 4. Semi-logarithmic plots of reaction rates k_I (●) and k_{II} (■) of mechanisms (I) and (II) in H_2O/DMSO solvent mixtures of molar fraction x_{DMSO} (Symbols o and □ refer to corrected values, see the text).

k_I values are roughly disposed along a horizontal line D_I. Deviations are observed for mixtures A and C, this is probably due to the underestimated value of the pK of the 4-nitrophenol used as a pH indicator in these media (see above). In effect, it seems unconceivable that the rate of reaction (I) may be decreased by about an order of magnitude in B (compared to A), in spite of the well-known increase of basicity of the attacking hydroxide ion OH^- by addition of DMSO. The deviations of log k_I (by 1.0 and 0.5 units for solvents A and C, respectively) may be assumed to represent the error δ_{pK} between the quoted values (8.3 and 9.0 respectively), and the true values, thus estimated as pK_{NH} = 9.3 and 9.5, respectively. This correction would raise the k_I values themselves to 3.1 and 2.3×10^9 $M^{-1}s^{-1}$ in A and B, as noted in Table 4.

This allows in turn to bring the same correction to rate constant in solvent C, $k_{II} = 1.94 \times 10^8$ $M^{-1}s^{-1}$. The set of four points representing the k_{II} values obtained in solvents C,D and DMSO, and in solvent E (see below) are approximately located along a straight line D_{II} of negative slope (If the linearity is assumed to hold for solvent B, we may infer a rate constant $k_{II}^{(B)} \sim 5 \times 10^8 M^{-1}s^{-1}$). This means that rate constant k_{II} is progressively decreasing from water to DMSO, while k_I is kept constant. In spite of this fact, mechanism (II) is predominant only in the less aqueous systems, because of the much sharper increase of pK_{SH} compared to pK_{NH}.

Line-broadening of the water resonance

A first experiment was carried out in solvent D. The NMR exchange rate $1/\tau'_{PH}$ was again found proportional to 1/[H+] with a coefficient $k_{obs} = 1.747 \times 10^{-12} M.s^{-1}$, as shown by the straight line (D) observed in a logarithmic plot of $1/\tau'_{PH}$ versus pH (Fig.5).

The value of k_{obs} is clearly larger than expected for mechanism (I) alone, in which case k_{obs} shoud be equal to $k_I = 0.117 \times 10^{-12}$ $M.s^{-1}$ and the straight line D of figure 5 should coïncide with line $D_{(I)}$ of equation : $\log 1/\tau'_{PH} = \log k_I + pH$. In fact, line D seems rather to be in the continuation of the line joining the points representing $1/\tau_{PH}$, the NMR rate constant deduced from the coalescence of the α-methylenic protons (see above, Table 3), in another pH range. This indicates that k_{obs} should refer to the sum of mechanisms (I) and (II), in which case k_{obs} should be equal to $k_I + k_2C = 1.42 \times 10^{-12} M.s^{-1}$. The deviation of *ca.* 30 % between the experimental and calculated values is probably within the uncertainty ranges accumulated in the determination of $\Delta\nu_{1/2}$, pK_{SH} and pK_{NH}.

This means that reaction (II) should involve water molecules intervening to facilitate proton transfer between the amide bond and the amide anion according to the scheme:

$$PH + \underset{\underset{H}{|}}{(O\text{-}H)_n} + P^- \xrightarrow{k} P + \underset{\underset{H}{|}}{(H\text{-}O)_n} + HP \qquad (II')$$

where $n \geq 1$. For a given H_2O/DMSO mixture, the overall rate constant k_{II} includes the water concentration $k_{II} = k \cdot [(H_2O)_n]$, this explains, among other causes, the decrease of k_{II} in the less aqueous mixtures.

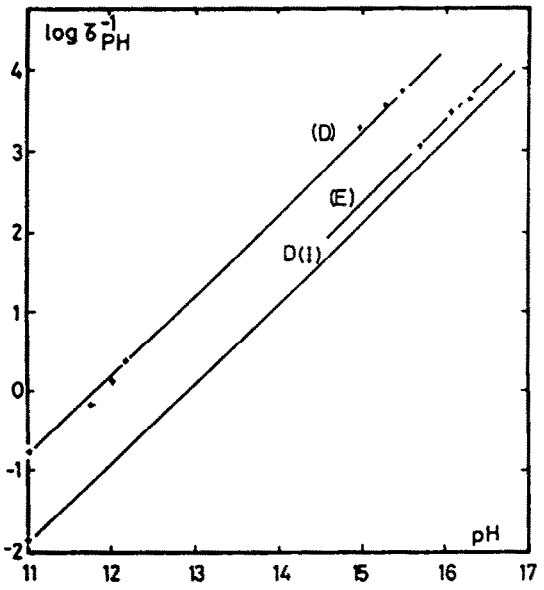

Fig. 5. Semi-logarithmic plots of NMR rate constants *versus* the pH, (a) $1/\tau_{PH}$ deduced from the line-brondering of the water resonance) for solutions D and E (lines D and E, respectively), and (b): $1/\tau_{PH}$ (deduced from the α-methylenic lines of the cyclopeptide) for solution D only (Line D). Line D is compared to line D(I) representing the contribution of mechanism (I) alone.

The intervention of bridging water molecules in proton transfers within an acid-base pair has been introduced by Grunwald *et al* [18] in the case of methylammonium salts and the conjugate amines. Mechanism (II') has proved to be of a great generality, and is predominant over mechanism (II) when the protonation-deprotonation process occurs between sterically hindered sites, as in 2,2,6,6-tetramethylpiperidine derivatives[19] or in nucleic acid-base pairs[20]. In the case of piperidinic substrates[19], we have shown a large decrease, by *ca.* three orders of magnitude, of rate constants k_{II} by going from water to DMSO. We have a somewhat analogous situation here, where a decrease of k_{II} by a little less than three orders of magnitude is observed from solution B to anhydrous DMSO.

A second experiment was then performed in solvent E, in which the observation of α-methylenic protons is impossible. Experimental data are again consistent with a direct proportionality of $1/\tau'_{PH}$ to $1/[H^+]$ with a coefficient $k_{obs} = 2.01 \times 10^{-15} M.s^{-1}$, as shown by the linear plot of log $1/\tau'_{PH}$ versus pH (line E in figure 5). In this case, mechanism (I) brings a negligible contribution $k_I \sim 10^{-15} M^{-1}s^{-1}$ to k_{obs} even if a constant maximum value of $\sim 10^{9} s^{-1}$ is assumed for k_I. We may therefore assign k_{obs} to mechanism (II') only, this allows us to compute the value of k_{II} in solvent E, $k_{II}^{(E)} = 3.58 \times 10^{6}$, which is clearly in line with the values obtained for solvents C, D and DMSO (Fig. 4).

pK measurements

The pK of cyclo-Glycyl-L-Prolyl in DMSO is slightly smaller, by *ca.* one pK unit, than the one[2] of the linear dipeptide PG_2. This difference may be due to either the cyclic conformation of the peptide presently investigated, or to the influence of the protecting groups in PG_2. Nevertheless this confirms a pK of *ca.* 18-19 for the peptide bond in DMSO[2].

pK measurements show a nearly constant value in the less aqueous mixtures, from solvent D ($x_{DMSO} = 0.700$) to pure DMSO ($pK_{NH} = 18.3$). This constancy probably reveals a preferential solvation of the peptide bond and of the hydrogen and amide ions by DMSO rather than by water. For more aqueous systems,the pK seems to decrease, $pK_{NH} = 16.6$ in mixture C. The value in water, although unknown, should be around 13, if we admit, as for NMA[1], a *ΔpK* of transfer from DMSO to H_2O of about 5.

The knowledge of both k_I and K_{NH} in solvents C and D allows us to compute the backward rate constant k_{-I} of reaction (I):

$$P^- + H_2O \rightarrow PH + OH^-$$

since $k_{-I} = k_I K_{SH}/K_{NH}$ (4)

Orders of magnitude of 10^5 and $10^6 M^{-1}s^{-1}$ are obtained for k_{-I} in C and D (Table 4). These values are far from the diffusion limit, which is often considered to be reached for reprotonation of such strong bases as amide anions by water molecules. This is again in favour of strong specific solvation of the amide anion by DMSO preventing the approach of water molecules. Another explanation is that the water molecules themselves are less available in mixtures containing a large amount of DMSO, because of strong associations between DMSO and H_2O molecules, a phenomenon often invoked in the literature[21]. If the diffusion-limit is assumed to be effectively reached for the backward and forward reactions in equibrium :

$$PH + OH^- \underset{k_{-I}}{\overset{k_I}{=}} P^- + H_2O$$

then $K_{NH} = K_{SH}$ according to eqn.4. On the contrary, in less aqueous media, K_{NH} becomes larger than K_{SH}, this requires that k_{-I} is continuously decreasing, as k_I remains nearly constant.

CONCLUSIONS

These measurements give some explanation about the switch of mechanism (I) to mechanism (II) from water to DMSO. The forward reaction in mechanism (I) is nearly constant because the diffusion limit is already reached in pure water as a consequence of a sufficient acidity of the peptide bond (this was not the case for NMA, where $k_I^{H2O}(NMA) = 1.56 \times 10^6 M^{-1}s^{-1}$). Rate constants k_I are levelled to their diffusion limit on further additions of DMSO which are accompanied by an increasing basicity of the hydroxide ion. On the contrary, the backward reaction in mechanism (I) continously decreases from H_2O to DMSO, thus ensuring $pK_{NH} << pK_{SH}$ on the DMSO side of the binary solvent mixtures. This inequality, which is equivalent to $[P^-] >> [S^-]$ explains why mechanism (I) becomes uneffective in the less aqueous solvents, in spite of the fact that $k_I > k_{II}$.

In pure DMSO, mechanism (II) alone is effective, and is relatively slow, contrary to the case of NMA, where k_{II} has reached its diffusion limit. Addition of water increases k_{II} mainly beacuse of the intervention of water molecules transferring protons along a Grotthus type chain. k_{II} reached values close to the diffusion limit for the more aqueous solvents (Fig.4), in which however the contribution of mechanism (I) to the overall proton transfer becomes negligible.

In $DMSO/H_2O$ mixtures often used for NMR studies of peptides, both types of exchanges are slow[22] in the absence of any added base, since both the solvent and the peptide are poorly self-ionized. Slightly basic $DMSO/D_2O$ mixtures have been used to characterize[14,22-24] strong internal or loose intermolecular hydrogen bonds in polypeptides by observing slow or fast NH-ND exchange rates, respectively. Under these conditions, the NH-ND exchange thus involves the penetration of a continuous chain of hydrogen-bonded water molecules up to the exchanging peptide bond, the basic partner at the other end of the chain being any strong base in the medium: hydroxide ions (which may as well operate alone) and amide anions in the present investigations.

Acknowledgements

All NMR spectra were recorded on spectrometers of the NMR Center of the University of Nancy. The authors thank Mrs. Eppiger for her technical assistance and Dr. Serratrice for the 2D COSY spectrum.

BIBLIOGRAPHY

1 J. Chrisment, J.-J. Delpuech, W. Rajerison, *J. Chim. Phys.*, **1983**, 80, 747.
2 J. Chrisment, J.-J. Delpuech, W. Rajerison, C. Selve, *Tetrahedron*, **1986**, 42, 4743.
3 R. Gaboriaud, *J. Chim. Phys.*, **1968**, 1155.
4 R. Gaboriaud, *Ann. Chim.*, **1967**, 201.
5 J.C.Hallé, R. Gaboriaud, R. Schaal, *Bull. Soc. Chim.*, **1969**, 1851.
6 J.C. Hallé, R. Gaboriaud, R. Schaal, *Bull. Soc. Chim.*, **1969**, 2047.
7 J.C. Hallé, R. Schaal, *Bull. Soc. Chim.*, **1972**, 3785.
8 R. Gaboriaud, J.C. Hallé, P. Letellier, *Bull. Soc. Chim.*, **1975**, 1093.
9 J. Chrisment, J.-J. Delpuech, *J. Chem. Soc. Perk. II*, **1977**, 407.
10 J. Chrisment, D. Nicole, J.-J. Delpuech, *C.R. Acad. Sc. Paris*, **1978**, 286C, 541.
11 R.S. Stearns, G.W. Wheland, *J. Amer. Chem. Soc.*, **1947**, 62, 2025.
12 F. Rodante, *Thermochimica Acta*, **1978**, 23, 321.
13 A. Binder, S. Ebel, *Arch. Pharmaz.*, **1975**, 308, 727.
14 K. Wüthrich, "NMR in Biological Research: Peptides and Proteins", North-Holland, Amsterdam **1976**.
15 K. Wüthrich, "NMR in Proteins and Nucleic Acids", Wiley, New York **1986**.
16 S. Meiboom, *J. Chem. Soc.*, **1961**, 34, 375.
17 J.-J. Delpuech, F. Siriex, M.N. Deschamps, *Org. Magn. Resonance*, **1972**, 4, 651.
18 E. Grunwald, A. Loewenstein, S. Meiboom, *J. Chem. Phys.*, **1957**, 27, 630.
19 B. Bianchin, J. Chrisment, J.-J. Delpuech, M.N. Deschamps, D. Nicole, G. Serratrice, in "Protons and Ions Involved in Fast Dynamic Phenomena", Proceedings of the 30th International Meeting of the Société de Chimie Physique, P. Laszlo Ed., Elsevier, **1978**, 265.
20 M. Guéron, M. Kocloyan, J.-L. Leroy, *Nature*, **1987**, 328, 89.
21 D. Martin, in "Dimethyl Sulphoxide", D. Martin, H.G. Hauthal, E.S. Halberstadt Eds., Van Nostrand, **1975**, 61.
22 L.C. Craig, D. Cowburn, H. Bleich, *Annu. Rev. Biochem.*, **1975**, 44, 477, and references therein.
23 N. Rama Krishna, D.H. Huang, J.D. Glickson, R. Rowan, R. Walter, *Biophys. J.*, **1979**, 26, 345.
24 S.L. Laiken, M.P. Printz, L.C. Craig, *Biochemistry*, **1969**, 8, 519.