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Ligand exchange reactions of proton bound dimers of carboxamides models for protonated proteins

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Dedicated to Prof. J.L. Beauchamp on the occasion of his 60th birthday.

Abstract

The kinetics of the ligand exchange reaction of the proton bound homodimer of primary amides propionamide (PPA) and pivalamide (PVA), of the tertiary amide *N*,*N*-dimethylformamide (DMF), and of the mixed proton bound heterodimer of DMF with *n*-propyl amine using 14 reactants were investigated by FT-ICR spectrometry. The proton bound dimers of the amides undergo sequential exchange of both ligands if a more basic amide or another strongly polar reactant is used. With amines and other reactants of small polarity the exchange of only one amide ligand is observed. The proton bound heterodimer of DMF with *n*-propyl amine exhibits role specificity for the two different ligands: the DMF ligand is selectively exchanged by polar reactants while the *n*-propyl amine is selectively exchanged by a more basic amine. This behavior is understood by a "solvation model" for the structure of such heterodimers. The reaction efficiency of an exothermic ligand exchange by a polar reactant is always large. This is explained by a profound electrostatic activation of the initial encounter complex which drives the exchange reaction. Exothermic ligand exchanges of the proton bound dimers of primary amides by amines are moderately efficient, while the same exchanges of the proton bound dimer of the tertiary DMF are slow and inefficient processes. This different behavior is explained by different mechanisms of the ligand exchange. In the case of dimers of primary amides the incoming amine is initially bonded to an acidic H atom of the amino group of the amide, and the exchange proceeds by a proton shift via a relay mechanism, while in the case of dimers of tertiary amides the exchanging amine has to approach the proton of the proton bridge directly in a perpendicular mode. This mechanism explains the severe steric hindrance observed for the ligand exchanges. The significance of these results for the reaction of protonated proteins in the gas phase is briefly discussed. (Int J Mass Spectrom 222 (2003) 27–40)

Keywords: Proton bound dimers; Clusters; Amide; Ion–molecule reaction; Ligand exchange; Kinetics; FT-ICR spectroscopy

1. Introduction

Many properties of proteins and peptides depend on their ability to acquire intra- and intermolecularly hydrogen bonds and proton bonds, which involve peptide bonds and other functional groups like amino groups or carboxylic groups of amino acid residues of the protein. In particular, the charged and presumably stronger proton bond between peptide bonds and basic functional groups is of importance for the enzymatic activity of proteins, which frequently is accompanied by proton release to a proton bound substrate [1]. Further, hydrogen bonds and proton bonds

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make a fundamental contribution to the stability of the tertiary structure of a protein, and changes of the conformation of the protein are associated with the breaking and making of hydrogen bonds and proton bonds within the macromolecule. Protonated peptides and proteins can be prepared in the diluted gas phase of a mass spectrometer by the almost omnipotent ionization techniques matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). Hence, the analysis of peptide radical cations of the early days of peptide mass spectrometry [2] has been replaced by studies of protonated peptides and proteins using a multitude of mass spectrometric techniques. Following with interest the development of peptide mass spectrometry by many research groups for now 40 years has resulted in an impressive tableau of fascinating studies and results, not only of the application of mass spectrometry to biochemistry and medical sciences, but also of more fundamental studies to perceive the structures and reactions of protonated peptides and proteins in the gas phase [3]. The implications of proton bridges and "mobile protons" for the properties of protonated peptides in the gas phase are obvious [4]. For instance, the most important backbone cleavage of a protonated peptide for sequencing requires a peptide bond protonated at the amide-N atom. Nonetheless, it is known that an amide or peptide bond is preferentially protonated at the carbonyl-O atom [5], but up to now there is no definite mechanisms known how the proton is transported to the N atom [6]. Further, the conformation of a protonated protein as well as its reactivity in H/D exchanges with external H(D) donors is undoubtedly effected by internal hydrogen and proton bonds. This has been especially demonstrated by Beauchamp and coworkers [7] in keynote papers revealing basic mechanisms for intra- and intermolecular interactions of functional groups of a protonated protein.

Although a careful mass spectrometric investigation of especially designed peptides and proteins is indispensable to make progress in the understanding of proton-induced reactions of these biomolecules, in view of the complexity of these macromolecules and their reactions considerable support can also be obtained by a study of more elementary model systems. In this context, a study of the protonation of amides and the reactions of their proton bound dimers and clusters is of particular interest since the amide group is equivalent to the peptide bond as the principal functional group of proteins. Theoretical calculations indicate that in peptides the preferred site of protonation is not only an amino group at the N-terminus or at a side chain of an amino acid residue but also the formation of a proton bridge between carbonyl-O atoms of appropriately oriented peptide bonds [4,8]. This situation can be simulated by an internal proton bridge in protonated diamides with a predicted orientation of the amide groups fixed by the carbon frame of the diamide molecule or modeled by proton bound homo- and heterodimers between simple amide molecules and between an amide and an amine molecule [9]. In this model system, "proton hopping" between functional groups within a protonated protein turns into a ligand exchange of the proton bound dimer, which can be conveniently followed by FT-ICR spectrometry. In a previous paper, we have reported the results of a study of ligand exchange reactions of the proton bound homodimer [DMF·H^+·DMF] of *N*,*N*-dimethylformamide (DMF) with other amides and amines under reversible reaction conditions, which result either in equilibria between all or only between specific proton bound dimers of the system [10], depending on the chemical nature of the reactant. In this paper the results of an investigation of the kinetics of the ligand exchange reaction of proton bound homo- and heterodimers of amides and amines are presented. It was shown in the previous equilibrium studies that amides as strongly polar ligands and amines as less polar or non-polar ligands behave differently in proton bound dimers. This is substantiated in this paper by the kinetics of the ligand exchange reactions involving polar and non-polar reactants. In addition to [DMF·H^+·DMF], the reactions of the proton bound dimers [PPA·H^+·PPA] of propionamide (PPA, CH_3CH_2–$CONH_2$) and [PVA·H^+·PVA] of pivalamide (PVA, $(CH_3)_3C$–$CONH_2$) as examples of proton bound dimers of primary amides were studied. Finally, the heterodimer [DMF·H^+·*n*-propyl amine]

was chosen as an example of a proton bound dimer containing an amide and amine. It will be shown that each of these different types of proton bound dimers of amides exhibit their own chemistry.

2. Experimental

The amide DMF as well as the amines and the other reactants used in this study are commercially available as pure compounds (purity of all compounds >99%) and were used without further purification. PPA and PVA (trimethyl-acetamide) were prepared from the corresponding acyl chlorides and NH_3 using standard chemical procedures [9] and were obtained by recrystallization as chromatographically pure compounds with the correct physical and spectroscopic properties.

A Bruker Spectrospin CMS47X FT-ICR spectrometer was used for all experiments. This instrument is equipped with an external electron impact ionization (EI) ion source and a chemical ionization (CI) ion source, a 4.7 T superconducting magnet [11], and an Infinity® ICR cell [12]. The temperature of the external CI ion source was about 200 °C while the ICR cell was kept at room temperature during all experiments. To prepare proton bound homodimers of the amides these were introduced into the CI ion source by the heated reservoir of the inlet system for liquids and a temperature controlled probe for solid samples, respectively. Isobutane was used as the CI gas at a pressure of 6–15 mbar, and primary ionization of the CI plasma was achieved by 20–30 eV electrons. The ions formed in the external CI ion source including protonated monomers and proton bound clusters were focused for 50–100 ms into the Infinity® ICR cell and trapped using a trapping voltage of 1 V. All ions besides that selected for the experiment were eliminated from the ICR cell by an ion ejection procedure. This includes a broad band rf pulse (broad band ejection) of about 80VP-P and a length 60–80 ms for ions with *m*/*z* values sufficiently different from that of the selected ion which was followed by a series of single rf pulses of appropriate frequency (single shots) with 10–15VP-P and a length of 1.5–3.5 ms for ions with *m*/*z* values close to that of the selected ion. Next, the trapped ions were cooled by collision with Ar atoms introduced into the cell by three to five subsequent pressure pulses of 5 ms duration and a pressure of Ar of 10 mbar in the gas reservoir of the inlet system.

After a delay time of about 200 ms following the cooling period the reaction of the selected ion with the neutral reactant present in the ICR cell at an appropriate constant background pressure was observed by taking mass spectra at different reaction times and constructing therefrom the curves of the dependence of the relative ion intensity on reaction time (kinetic plots) for reactant ion and product ions. The reaction was followed for at least 20 s and an excellent pseudo-first-order kinetic was observed in all cases. The pseudo-first-order rate constant, k_{exp}, was calculated from the kinetic plot using the curve fitting facilities of the Origin program [13]. The bimolecular rate constant k_{bi} was derived from k_{exp} by taking into account the number density of the neutral reactant calculated from its partial pressure in the ICR cell. The neutral reactants for the ligand exchange reaction were introduced into the ICR cell from a batch inlet system with precision valves and using carefully degassed liquid samples. The pressure within the ICR cell was obtained from the reading of the ionization gauge positioned between the ICR cell and the high vacuum pump of the cell. The reading of this ionization gauge was calibrated by determination of the well established rate constant of the reaction $NH_3^+ + NH_3 \rightarrow NH_4^+ + NH_2$ [14]. Further, the appropriate compound-specific corrections for the sensitivity of the ionization gauge were used [15]. The reproducibility of the kinetic FT-ICR experiments was very good, and the main error of the rate constant k_{bi}, which was estimated to be ±30%, results from the error of measurement of the partial pressure of the neutral component. It is convenient to use the reaction efficiency, eff, instead of the rate constant k_{bi} in discussing the rates of ion–molecule reaction. Here eff is defined as the percentage of collisions which result in a observable chemical change of the reactants and is calculated from the rate constant k_{bi} and the collision

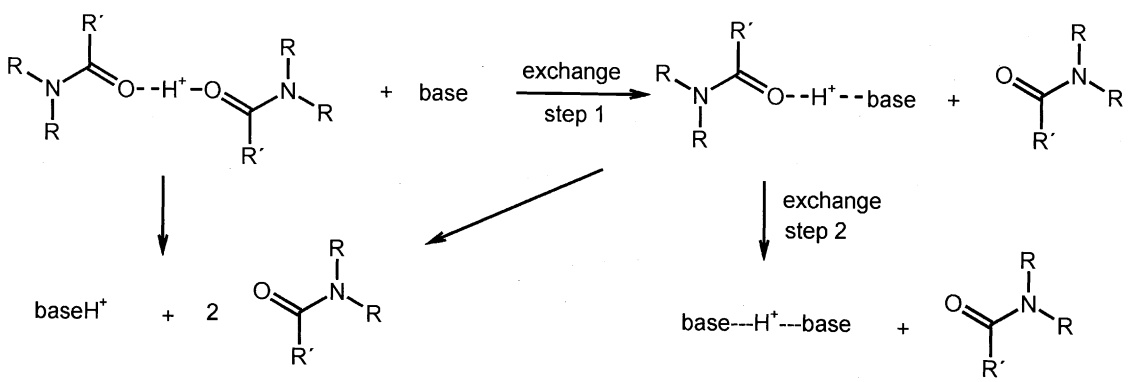
rate constant k_c by eff $= 100 \times k_{bi}/k_c$ (%), where k_c is obtained by the method of Su and Bowers [16].

3. Results and discussion

3.1. Ligand exchange of the proton bound dimer [PPA·H$^+$·PPA] and [PVA·H$^+$·PVA]

The proton bound homodimers of PPA and PVA (trimethylacetamide) are readily formed with high abundance under CI conditions in the external ion source of the FT-ICR spectrometer. If these proton bound homodimers are focused into the gas phase of the FT-ICR cell which contains only one suitable base, one or both amide ligands of the proton bound dimer may be exchanged by a two-step process by molecules of the base and/or the proton bound dimer may decompose by transfer of the proton to a single base molecule (Scheme 1). Since only the base is present in large excess in the neutral gas phase of the FT-ICR cell, all these processes are essentially irreversible and should follow the kinetics of a pseudo-first-order reaction. However, which of these processes actually occurs depends on the difference of the gas phase basicity (GB) or proton affinity (PA) of the amide and the respective base, and on the chemical nature of the reactants.

Tables 1 and 2 summarize the results of the kinetic measurement of the ligand exchange reaction of [PPA·H$^+$·PPA] and of [PVA·H$^+$·PVA] with a set of 14 ligands, mostly amines and amides, and Figs. 1 and 2 display kinetic plots for the reaction of [PPA·H$^+$·PPA] with methyl amine and *N*,*N*-dimethylacetamide, respectively, as examples of the two types of ligand exchange reactions observed with these systems. The reaction of [PPA·H$^+$·PPA] with methyl amine results in the exchange of only one PPA ligand besides a small amount of proton transfer to methyl amine. Contrasting, the reaction of [PPA·H$^+$·PPA] with *N*,*N*-dimethylacetamide occurs by substitution of both PPA molecules and converts the original proton bound homodimer in two steps of similar reaction efficiencies into the new proton bound homodimer of *N*,*N*-dimethylacetamide. Again some proton transfer to *N*,*N*-dimethylacetamide is observed as a minor reaction. This different behavior, exchange of only one ligand and exchange of both ligands in a two-step process, is characteristic for the exchange reactions of proton bound homodimers of carboxamides by amines as not very polar reactants ($\mu_D < 1.5$ D) and by amides as strongly polar reactants ($\mu_D > 3$ D), and corroborates the results obtained earlier by equilibrium studies of proton bound dimers of carboxamides and amines [10]. That the reason for this different behavior is indeed the dipole moment of the reactant is seen by the reaction of pyridine which exhibits a distinct dipole moment of 2.25 D and exchanges also both ligands of the proton bound homodimers of amides with a noticeable efficiency. It is of interest to note that the reaction of a proton bound homodimer of an amide with an amine



Scheme 1.

Table 1
Reaction efficiency of the first (eff(1st step)) and second (eff(2nd step)) ligand exchange and of proton transfer (eff(H^+)) of the proton bound homodimer of propionamide [PPA·H^+·PPA][a]

Reactant	PA(reactant) (kJ/mol)[b]	μ_D(reactant) (Debye)[c]	eff(1st step) (%)	eff(2nd step) (%)	eff(H^+) (%)
N-Methylformamide	851.3	3.84	9	1.3	0.2
Ammonia	853.6	1.47	0.02	<0.01	<0.01
Acetamide	863.6	3.90	18	4.3	0.3
Di-*s*-butyl ether	865.9	1.20	0.18	<0.01	0.02
DMSO	884.4	3.90	27	21	3
DMF-(D_7)	887.5[d]	3.86	77	57	6
Methyl amine	899.0	1.29	54	<0.01	5
N,*N*-Dimethylacetamide	908.0	3.81	42	38	3
Ethyl amine	912.0	1.22	48	<0.01	3
n-Propyl amine	917.8	1.18	38	0.05	3
Pyridine	930.0	2.25	52	20	11
Trimethyl amine	948.9	0.63	49	<0.01	7
Piperidine	954.0	1.19	35	3	14
Tri-*n*-propyl amine	991.0	0.74	37	>0.01	21

[a] PA(propionamide) = 873 kJ/mol from [18].
[b] From NIST Standard Reference Database No. 69—release July 2001 (http//webbook.nist.gov./chemistry).
[c] From [24].
[d] PA of DMF.

under the conditions of irreversible reaction does not produce a proton bound trimer containing a molecule of the attacking amine and the two molecules of the amide of the initial homodimer, which is observed under reversible reaction conditions where the neutral gas phase contains the amide besides the amine [10]. Evidently the formation of the trimer occurs by a two-step process, in which the first step generates

Table 2
Reaction efficiency of the first (eff(1st step)) and second (eff(2nd step)) ligand exchange and of proton transfer (eff(H^+)) of the proton bound homodimer of pivalamide [PVA·H^+·PVA][a]

Reactant	PA(reactant) (kJ/mol)[b]	μ_D(reactant) (Debye)[c]	eff(1st step) (%)	eff(2nd step) (%)	eff(H^+) (%)
N-Methylformamide	851.3	3.84	1.3	0.2	0.05
Ammonia	853.6	1.47	<0.01	<0.01	<0.01
Acetamide	863.6	3.90	5.4	1.0	0.1
Di-*s*-butyl ether	865.9	1.20	0.3	0.01	0.05
DMSO	884.4	3.90	23	9	1
DMF-(D_7)	887.5[d]	3.86	49	45	4
Methyl amine	899.0	1.29	56	<0.01	4
N,*N*-Dimethylacetamide	908.0	3.81	61	51	3
Ethyl amine	912.0	1.22	47	<0.01	4
n-Propyl amine	917.8	1.18	37	0.12	7
Pyridine	930.0	2.25	52	11	8
Trimethyl amine	948.9	0.63	49	<0.01	6
Piperidine	954.0	1.19	43	1.1	7
Tri-*n*-propyl amine	991.0	0.74	39	<0.01	15

[a] PA(pivalamide) = 888 kJ/mol from [19].
[b] From NIST Standard Reference Database No. 69—release July 2001 (http//webbook.nist.gov./chemistry).
[c] From [24].
[d] PA of DMF.

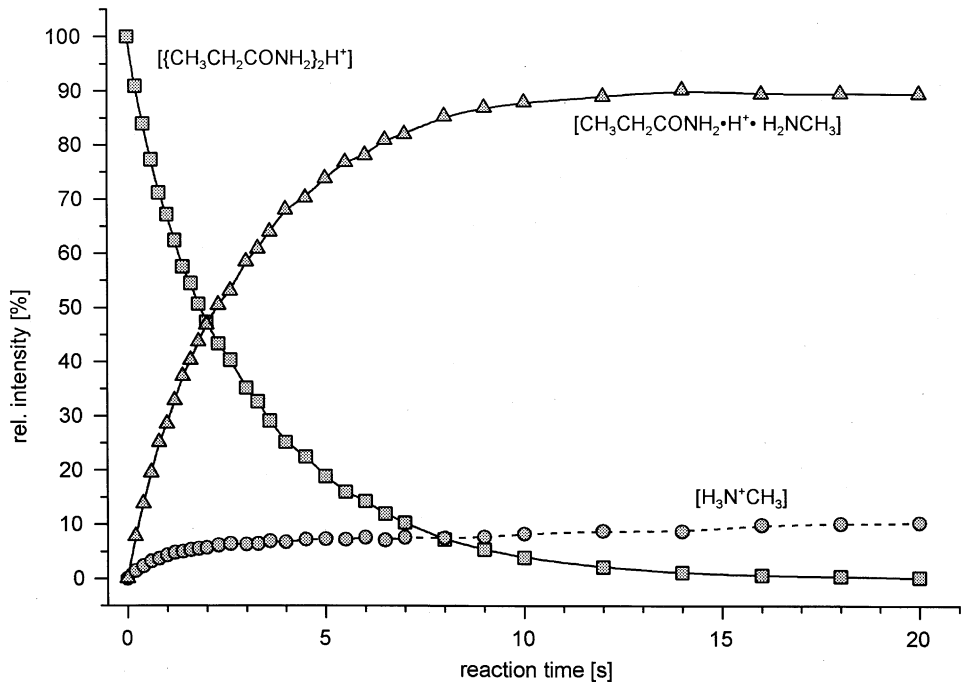


Fig. 1. Kinetic plot of the reaction of the proton bound homodimer of propionamide and methyl amine.

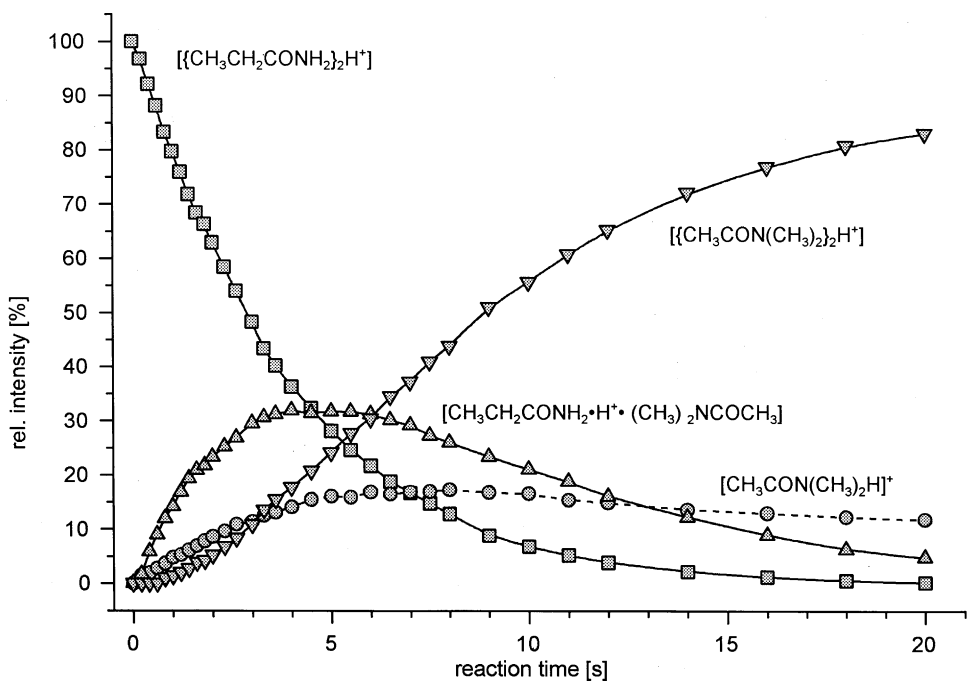


Fig. 2. Kinetic plot of the reaction of the proton bound homodimer of propionamide and dimethyl acetamide.

a heterodimer by exchange of one amide ligand and the second step completes the trimer by attachment of the second molecule of amide. However, in principle a proton bound trimer [amide·amineH^+·amide] could arise additionally by a single step insertion process of the amine into the proton bridge of the proton bound dimer [amide·H^+·amide]. Such a mechanism has been suggested by Lifshitz and coworkers [17] for the formation of proton bound trimers in the reaction of ammonia or amines with proton bound dimers of aliphatic nitriles and of ethers exhibiting a significant dipole moment. The absence of the trimer [amide·amineH^+·amide] in the present case excludes definitely this mechanism for the reaction of proton bound dimers of amides, and makes this mechanism also questionable for the reaction of other proton bound dimers of polar compounds.

The data of Tables 1 and 2 show that the reaction efficiency for the exchange of the first ligand of the homodimers [PPA·H^+·PPA] and [PVA·H^+·PVA] spans from <0.01 to >60%. The larger values are found for reactants with PA(ligand) > PA(propionamide) = 873 ± 5 kJ/mol [18]. The first ligand exchange becomes exothermic if the molecular pair proton affinity (MPPA) of the molecular pair (PPA; ligand) or (PVA; ligand) exceeds the MPPA(PPA; PPA) or MPPA(PVA; PVA). These latter values can be calculated from the PA(PPA) or PA(PVA) and the bond dissociation energy (BDE) of the proton bound homodimer [PPA·H^+·PPA] or [PVA·H^+·PVA]. These values have been determined previously [19], yielding MPPA(PPA; PPA) = 980 ± 10 kJ/mol and MPPA (PVA; PVA) = 1001 ± 10 kJ/mol. The increased MPPA(PVA; PVA) is mainly due to the increase in PA(PVA). The MPPA of (PPA; ligand) and (PVA; ligand) are not known, but it has been shown in the case of proton bound dimers of DMF [10] that MPPA(DMF; ligand) increases approximately by 0.45 × ΔPA of the difference of PA(DMF) and PA(ligand). Since a similar effect of PA is expected also for other amides, every ligand more basic than PPA or PVA will undergo an exothermic ligand exchange as observed experimentally. At a first glance it appears that there is a considerable scattering of the data which obstructs any simple correlation of efficiency of the ligand exchange with increasing PA. However, besides PA other effects of the ligand structure must influence the reaction efficiency. One important property of the reactant is the dipole moment. This is most clearly seen for reactants which are less basic than PPA or PVA (data at the top of Tables 1 and 2). Thus, PA(*N*-methylformamide) and PA(acetamide) are smaller than PA(NH_3) and PA(di-*s*-butyl ether), respectively, but nonetheless the ligand exchange by the polar amides is much more efficient. The results of the study of equilibria between proton bound dimers of amides, amines, and some selected compounds has shown, that the MPPA of an amide and a polar compounds is about 20 kJ/mol larger than that including an amine ligand of the same PA [10]. Thus, the increased reaction efficiency of a ligand exchange by polar reactants observed reflects obviously a more exothermic (or less endothermic) reaction for polar compounds. A second effect is a steric hindrance of the ligand exchange by bulky reactants. This effect becomes apparent in Tables 1 and 2 by the slightly decreased reaction efficiency of tri-*n*-propyl amine, although this amine is the most basic ligand studied here. As will be shown in a forthcoming paper [20], steric hindrance of the ligand exchange becomes a main factor in the reactions protonated diamides with a more shielded environment of the proton bridge. The third effect is the number of acidic hydrogen atoms both at the amide-N atom and the amine-N atom, but this effect is more clearly seen in the reaction of proton bound dimers of tertiary amides discussed in the following section.

The mechanism of formation of the protonated base produced by proton transfer from the proton bound dimer to the reactant during the side reaction is not completely clear. The efficiency of the proton transfer is mostly rather small, as expected for endothermic proton transfer, and it cannot be excluded that a fraction of the protonated base arises by reaction of some proton bound dimers which still contain excess kinetic energy in spite of the cooling procedure. Nonetheless, there is a trend of an increasing efficiency of proton transfer with increasing PA of the reactant, and at least for the strong bases trimethyl amine, piperidine and

tri-*n*-propyl amine the shape of the corresponding intensity curve of the protonated base shows clearly that proton transfer occurs from thermalized proton bound dimers. Probably the ligand exchange reaction in these reaction systems is sufficiently exothermic to promote the dissociation of a fraction of the newly formed and excited proton bound heterodimer [amide·H^+·amine].

3.2. Ligand exchange of the proton bound dimer [DMF·H^+·DMF]

The kinetic plot of the ligand exchange reaction of the proton bound homodimer [DMF·H^+·DMF] with ethyl amine is shown in Fig. 3, and the efficiencies of the ligand exchange reaction for the set of 14 reactants are collected in Table 3. Once again an exchange of both DMF ligands of the homodimer is observed only for polar reactants, while reactants with a small dipole moment (amines, ethers) exchange only one DMF molecule. Further, proton transfer to the basic reactant is observed as a side reaction. With respect to these properties the proton bound homodimer of the tertiary amide DMF behaves analogous to those of the primary amides PPA and PVA. However, the difference in the exchange efficiencies for polar and non-polar reactants is much more pronounced, and with the exception of pyridine the exchange efficiency for all amines is surprisingly small. This becomes obvious by comparing the exchange efficiency of the same amine in the reaction of the proton bound homodimers of the primary amides discussed before, which are larger by one order of magnitude. This indicates that not only the ligand exchange by polar and non-polar reactants is different, but also the ligand exchange in proton bound dimers of primary amides and of tertiary amides proceeds by different mechanisms. Tri-*n*-propyl amine exhibits a particularly small exchange efficiency of only 0.83% in spite of a PA of 84 kJ/mol with DMF, and the exchange is not much more efficient than proton transfer. This low reactivity of tri-*n*-propyl amine is undoubtedly a consequence of a combination of a steric hindrance of the exchange reaction and the fact that neither DMF nor the tertiary amine exhibit H atoms at the amino group.

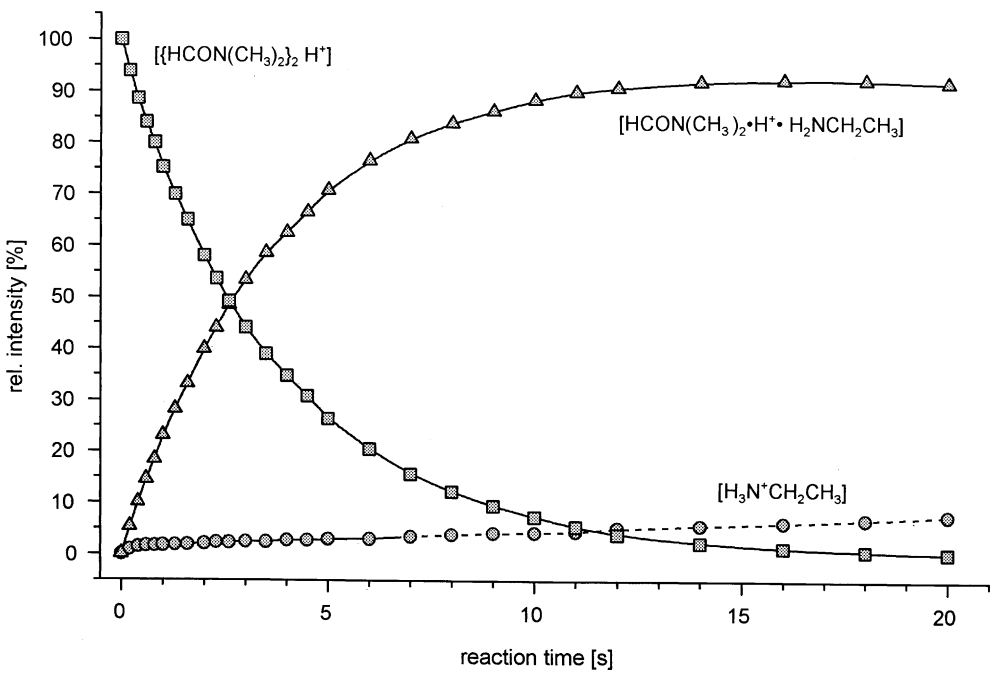


Fig. 3. Kinetic plot of the reaction of the proton bound heterodimer of *N*,*N*-dimethylformamide and ethyl amine.

Table 3
Reaction efficiency of the first (eff(1st step)) and second (eff(2nd step)) ligand exchange and of proton transfer (eff(H^+)) of the proton bound homodimer of [DMF·H^+·DMF][a]

Reactant	PA(reactant) (kJ/mol)[b]	μ_D(reactant) (Debye)[b]	eff(1st step) (%)	eff(2nd step) (%)	eff(H^+) (%)
N-Methylformamide	851.3	3.84	0.8	0.1	0.02
Ammonia	853.6	1.47	<0.01	<0.01	<0.01
Acetamide	863.6	3.90	1.8	0.2	0.04
DMSO	884.4	3.90	16.7	7.4	0.7
DMF-(D_7)	887.5[c]	3.86	40	24	3.0
Methyl amine	899.0	1.29	1.9	<0.01	0.1
N,*N*-Dimethylacetamide	908.0	3.81	47	35	4.3
Ethyl amine	912.0	1.22	4.6	<0.01	0.4
n-Propyl amine	917.8	1.18	4.7	0.01	1.5
Dimethyl amine	929.5	1.03	2.3	0.02	0.8
Pyridine	930.0	2.25	25	7.2	3.4
Trimethyl amine	948.9	0.63	2.0	0.04	0.4
Piperidine	954.0	1.19	6.4	0.5	2.8
Tri-*n*-propyl amine	991.0	0.74	0.8	<0.01	0.7

[a] PA values from NIST Standard Reference Database No. 69—release July 2001 (http//webbook.nist.gov./chemistry).
[b] From [24].
[c] PA of DMF.

3.3. Ligand exchange of the proton bound dimer [DMF·H^+·*n*-propyl amine]

The reaction efficiencies of the ligand exchange reaction of the proton bound heterodimer [DMF·H^+·*n*-propyl amine] with DMSO and *N*,*N*-dimethylacetamide as polar reactants and NH_3 and several amines as non-polar reactants are listed in Table 4. In all cases the exchange of only one ligand of [DMF·H^+·*n*-propyl amine] is observed, and proton transfer to the reactant is again a minor reaction pathway. Interestingly, the polar reactants DMSO and *N*,*N*-dimethylacetamide exchange selectively the polar DMF molecule and the amines substitute selectively the *n*-propyl amine ligand of [DMF·H^+·*n*-propyl amine]. The PA of DMSO and *N*,*N*-dimethylacetamide are equal or larger than PA(DMF), so that the exchange of the DMF by these reactants is exothermic and expected to be quite efficient. As anticipated an efficient exchange of *n*-propyl amine ligand in [DMF·H^+·

Table 4
Reaction efficiency ligand exchange and of proton transfer (eff(H^+)) of the proton bound heterodimer of DMF and *n*-propyl amine [DMF·H^+·*n*-propyl amine][a]

Reactant	PA(reactant) (kJ/mol)[b]	μ_D(reactant) (Debye)[b]	eff(1st step) (%)	eff(2nd step) (%)	eff(H^+) (%)
Ammonia	853.6	1.47	No reaction		
DMSO	884.4	3.90	<0.01	28	0.6
Methyl amine	899.0	1.29	2.2	<0.01	0.1
N,*N*-Dimethylacetamide	908.0	3.81	<0.01	54	3.2
Ethyl amine	912.0	1.22	20	<0.01	2.1
Pyridine	930.0	2.25	34	2	10.3
Piperidine	954.0	1.19	72	<0.01	10.8

[a] PA from NIST Standard Reference Database No. 69—release July 2001 (http//webbook.nist.gov./chemistry); PA(DMF) = 886 kJ/mol, PA(*n*-propyl amine) = 918 kJ/mol.
[b] From [24].

n-propyl amine] by an amine is only observed if the amine is more basic than *n*-propyl amine. Note however, that methyl amine, which is less basic than *n*-propyl amine but more basic than DMF, does neither substitute effectively *n*-propyl amine nor DMF. Obviously the polar ligand and the non-polar ligand in the mixed proton bound dimer [amide·H^+·amine] play a specific role in the exchange process.

3.4. Mechanisms of ligand exchange reactions in proton bound dimers of amides and amines

The results discussed in the previous sections clearly show that the course and the efficiency of the ligand exchange reaction of proton bound homodimers of amides and of heterodimers of amides and amines depends explicitly on the structures of the ligands and of the reactants. Although the reaction energy of the ligand exchange reaction plays an important role which is typical for gas phase ion–molecule reactions, the effects of ligand and reactant polarity and in particular the steric effect on the exchange efficiency is surprisingly large. This suggests that the structure of the proton bound dimers and the character of the proton bridge are different for the different classes of proton bound dimers, and that in these classes the ligand exchange reaction follows different mechanisms.

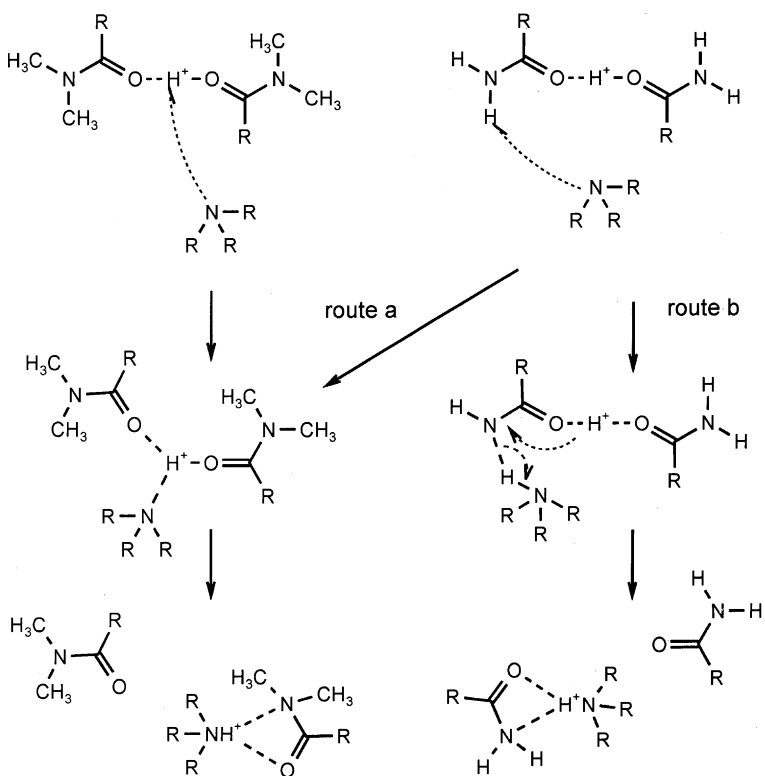
The positive effect of a strong dipole moment of the reactant on the efficiency of the ligand exchange reaction, which is observed for all types of proton bound dimers of amides, is easily explained by the typical reaction energy profile of a ion–molecule reaction in the diluted gas phase on the potential energy surface, which corresponds at least to a double well potential [21]. Generally, the first step of the reaction is the formation of an encounter complex driven by ion/dipole and ion-induced dipole attraction. In the diluted gas phase this generates an energetically excited complex. The excess energy of the complex depends on the magnitude of the ion/dipole forces and is used to overcome critical barriers separating reactants and products. The encounter complex of a proton bound dimer with a polar reactant molecule contains significantly more excess energy than a complex with a less polar reactant because of the stronger ion/dipole forces, and this results in an increased rate constant of the exchange reaction assuming similar critical energies and reaction energies in both cases. Thus, the different kinetic behavior of polar and non-polar reactants is a direct consequence of the theory of gaseous ion–molecule reactions [21]. However, more importantly this difference proves unambiguously that the ligand exchange reaction of proton bound dimers of amides does entail a critical barrier, in contrast to what is assumed for proton transfer reactions.

The role specificity of the ligands of the proton bound heterodimers [amide·H^+·amine], which allows exchange of the polar amide only by a polar reactant and exchange of the amine only by another amine, hints to a special structure of this heterodimer, in which the two ligands interact differently with the proton. In fact the behavior of proton bound heterodimers [amide·H^+·amine] during ligand exchange can be explained by a structure in which the ammonium ion is solvated by the polar amide molecule. In this case the exchange of the amide by another polar reactant corresponds to a switching of solvent molecules which is expected to be fast, while the exchange of the amine ligand by a more basic amine is practically an exothermic proton transfer, and the amide as the solvent molecule of the complex simply follows the migrating charge. Again this is expected to be a fast process. The reason for such a solvation complex structure for the dimers [amide·H^+·amine] is very likely that a polar ligand favors electrostatic bonding by ion/dipole forces which are maximal if the positive charge is concentrated in the protonated amino group and not delocalized in a proton bridge. This “solvation model” for proton bound heterodimers of amides and amines has already been introduced previously [10] to explain ligand exchange under the conditions of reversible reactions, where a proton bound trimer [$(amide)_2$·H^+·amine] is formed by association of a neutral amide molecule to the dimer [amide·H^+·amine]. However, the stability of the trimer [$(amide)_2$·H^+·amine] depends inter alia on the number of acidic hydrogens at the N atom of the amino group [9] showing that the “solvation model”

is an oversimplification and at least some additional bonding is exercised by hydrogen bridges in the complexes [amide·H^+·amine] and [$(amide)_2$·H^+·amine].

The number of acidic hydrogen atoms available in a proton bound homodimer [amide·H^+·amide] has a significant influence on the exchange efficiency of non-polar reactants. As discussed before, polar reactants surmount any critical barrier of the exchange process owing to the large electrostatic activation of the encounter complex. However, the clearly different exchange efficiency of a given amine towards the proton bound dimers of primary amides [PPA·H^+·PPA] and [PVA·H^+·PVA] and of the tertiary amide [DMF·H^+·DMF] shows that the critical energy for ligand exchange is systematically different for primary and tertiary amides. This could be the result of different structures for the proton bound dimers of primary and tertiary amides. It is sometimes assumed that a tertiary amide is protonated at the amide-N atom because of the electron releasing inductive effect of the N-alkyl groups. However, neither experiment nor theoretical calculations give any indication of N-protonation of amides [9]. Therefore, it is for sure that both primary and tertiary amides are protonated at the carbonyl-O atom. Further, all theoretical calculations show that the proton bound dimer of amides contains a proton bridge –C=O · · · H^+ · · · O=C– with the proton situated more or less symmetrically between the carbonyl-O atoms. Therefore, the different reactivity of proton bound dimers of primary and tertiary amides is due to different mechanisms of the ligand exchange reaction.

In the case of dimers of tertiary amides the amine undergoing exchange has to approach directly the proton of the bridge to replace one of the amide ligands in a nucleophilic substitution process (Scheme 2, route a). The incoming amine accesses the proton perpendicular to the proton bridge, and the critical



Scheme 2.

configuration of the ligand exchange process corresponds to a bifurcated proton bridge. This is a very different situation compared to the reaction path of a proton transfer between two *n*-bases, where ideally the critical configuration corresponds to a linear proton bridge between the two reactants. Likely a bifurcated proton bridge as the critical configuration is energetically more demanding, and because of the crowded situation at this bridge, the exchange process is expected to be sensitive to steric hindrance. The bulky dialkyl amino group of the tertiary amides may increase the steric effect, although these groups point away from the proton bridge. Such an inspection of the reaction path of the ligand exchange explains conclusively why even the exothermic ligand exchange of proton bound dimers of tertiary amides by an amine is a slow process, in particular for voluminous tertiary amines.

In principle, the same mechanism and the same reaction path could take place for proton bound dimers of primary amides. Nonetheless, a much more efficient ligand exchange reaction and less steric hindrance is observed experimentally for these dimers, if compared to dimers of tertiary amides. Evidently the incoming amine finds another way, which is energetically and sterically more favorable than the direct approach of the proton of the proton bridge, to replace a primary amide in a proton bound dimer. An obvious alternative is the initial docking of the amine at one of the acidic hydrogen atoms at the amide-N atom (Scheme 2, route b). Because of the association of the carbonyl-O atom of the amide in the proton bridge, the H atoms at the amide-N atoms are certainly more acidic than in a neutral amide, and the amino groups of the amide ligands of the proton bound dimer point outward and are easily accessible. Thus, binding of the amine at this position by a hydrogen bond is favorable even for bulky amines. Further, accepting the "solvation model" for proton bound heterodimers of amide and amines, the structure of the product of the ligand exchange reaction with an amide molecule bound mainly by electrostatic forces to an ammonium ion differs essentially from the structure of the initial proton bound dimer with a "true" proton bridge. Therefore, after destabilizing the proton bridge of the initial dimer by binding of the amine at one of the outer amino groups of the amides, a shift of protons gives rise to the ligand exchange product. This reaction path of the ligand exchange in proton bound dimers of primary amides corresponds to a proton transport by relay mechanism [7b] combined with an internal proton shift to avoid formation of the energetically not favored isoamide structure in the product dimer. It is reasonable to assume that such reaction pathway suffers less from steric hindrance, but evidently also the proton switching during the relay mechanism needs less critical energy than the formation of a bifurcated proton bridge.

4. Conclusion

The investigation of the kinetics of the ligand exchange reaction of proton bound homodimers of the primary amides *n*-propionamide PPA and PVA, of the tertiary amide DMF, and of the mixed heterodimer of DMF and *n*-propyl amine by amides or amines reveals an unanticipated dependence of the reactivity on the chemical nature of the dimer and the reactants. The results corroborate and extend earlier observations of ligand exchange equilibria between proton bound dimers of DMF and amides and amines [10]. The differences of the ligand exchange efficiencies of the classes of proton bound dimers and of strongly and weakly polar reagents demonstrate convincingly the existence of different structures for these proton bound dimers and different mechanisms of the ligand exchange. In particular, the structure of the proton bound heterodimer of PPA and *n*-propyl amine is better described as a complex of the *n*-propyl ammonium ion with PPA as solvent molecule bound by electrostatic forces. The loose structure of this complex allows efficient ligand exchanges, but both component exhibit a role specificity. The other proton bound dimers studied are composed of amide ligands only which are held together by a true proton bridge between the carbonyl-O atoms of the amides. An unexpected result for the ligand exchange of these proton bound dimers by amines is a distinct steric hindrance of the reaction which yields small exchange

efficiencies in particular for dimers of tertiary amides. The increased reactivity of the proton bound dimers of primary amides is attributed to a special mechanism in which the exchanging amine is initially attached to an acid H at the N atom of the amide and ligand exchange proceeds by proton switching in a relay mechanism, thus avoiding a direct attack of the sterically shielded proton of the proton bridge.

What is the significance of these results for proton shifts and conformational changes of protonated proteins? First it should be noted that the two types of proton binding discussed for the proton bound homo- and heterodimers of amides are also present in protonated proteins: the proton bridge between the carbonyl-O atoms of the amides corresponds to a proton bridge between the carbonyl-O atoms of different peptide bonds properly placed along the peptide chain, and the proton bound heterodimer [amide·H^+·amine] with the "amide-solvated ammonium ion" structure corresponds to the interaction of a protonated amino group at the N-terminus of the peptide chain or at the side chain of an amino acid residue with appropriately oriented peptide bonds. Recall that the two types of proton bonds exhibit different rate constants for ligand exchange. It is likely that this will be true also for the proton bridges in proteins, although the peptide bonds correspond to secondary amides with the exception of those of proline. Therefore, from our results one would predict that there should exist at least two classes of H/D exchanges with an external H/D donor observed in proteins: a fast one which involves the protons of solvated ammonium groups and a slower one for protons held in proton bridges between peptide bonds. This is of course known from experimental studies. The latter proton bonds are important features for tertiary structures of proteins, and since a change of the conformation of a protonated protein involves a ligand exchange of such proton bridges, one would expect also two categories of conformational changes: a fast one triggered by the quick movements of solvated ammonium ions and salt bridges in the polar environment of peptide bonds, and a slower one proceeding by breaking and restoring proton bridges between peptide bonds. In particular this latter process is sensitive to steric hindrance, and this may be one sensible explanation, why conformational changes of protonated proteins in the gas phase are indeed surprisingly slow [7,22,23].

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