Evidence by N-15 CPMAS and N-15-C-13 REDOR NMR for fixation of atmospheric CO2 by amino groups of biopolymers in the solid state

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · MAY 1999

Impact Factor: 12.11 · DOI: 10.1021/ja981747g

7 AUTHORS, INCLUDING:

Gerd Buntkowsky
Technical University Darmstadt
225 PUBLICATIONS **3,158** CITATIONS
SEE PROFILE

Jürgen-Hinrich Fuhrhop
Freie Universität Berlin
180 PUBLICATIONS **5,140** CITATIONS
SEE PROFILE

Hans-Heinrich Limbach
Freie Universität Berlin
333 PUBLICATIONS **8,854** CITATIONS
SEE PROFILE

Communications to the Editor

Evidence by ^{15}N CPMAS and $^{15}N-^{13}C$ REDOR NMR for Fixation of Atmospheric CO_2 by Amino Groups of Biopolymers in the Solid State

Volkmar Schimming, Christof-Gottfried Hoelger, Gerd Buntkowsky, Ingolf Sack, Jürgen-Hinrich Fuhrhop, Stefano Rocchetti, and Hans-Heinrich Limbach*

Freie Universität Berlin, Institut für Organische Chemie Takustrasse 3, D-14195 Berlin, Germany

Received May 19, 1998
Revised Manuscript Received March 29, 1999

Amino groups of organic and biological compounds can react with CO_2 in aqueous and nonaqueous environments to give the carbamates indicated in Figure 1.[1−4] The reaction is of importance in biological systems where it is used for the transport of CO_2.[3,4e,i,j] Mechanistic and pH-dependent studies indicate that ammonium groups have to be deprotonated before the reaction can occur. As HCO_3^- is preferentially formed at high pH,[4h] a maximum carbamate formation is observed at pH values corresponding to the pK_a values of the ammonium groups, i.e., for example 7 to 8 in the case of terminal amino groups of proteins,[4j] and pH 9 and 10 in the case of the α- and ϵ-amino groups of lysine.[4f]

However, to our knowledge, the reaction of amino groups of dry solids with atmospheric CO_2 under conditions of a reduced water content has not yet attracted attention. We have, therefore, studied this reaction using solid-state ^{15}N and ^{13}C NMR, in connection with cross-polarization (CP), magic angle spinning (MAS),[5] as well as $^{13}C-^{15}N$ REDOR (Rotational Echo Double Resonance) techniques.[6]

The compounds studied are α-ornithine-ω- ^{15}N-amino bola-amphiphile **1** and poly-l-lysine **2** (Figure 1). **1** was synthesized in a similar way as the lysine analogue.[7] The latter, **1**, forms spontaneously vesicular tubules by cooling micellar hot aqueous solutions at pH 10.5. Electron microscopy (Figure 1a) shows long tubules with monolayered membrane walls, where the amino headgroups are probably located inside the tubes and the polar tails outside (Figure 1a). The inner surface of the tubules is very large and thus favors solid-state reactions.

As the formation of tubular **1** strongly depends on pH, i.e., the state of deprotonation of the two amino and the carboxyl groups, we wanted to obtain more structural information concerning this problem using ^{15}N CPMAS spectroscopy.[9] Indeed, the spectrum of amorphous **1** lyophilized at pH 5 consists of a single line at about 5 ppm[10] (Figure 1b) which is typical for aliphatic $R-NH_3^+$ groups dissolved in water.[11] The sample did not show any alteration over a period of several weeks. By contrast, in the case of tubular **1** a shift to −13 ppm was observed (Figure 1c). This shift may be assigned either to a complete deprotonation of the ammonium group or to a water release and a partial deprotonation followed by the formation of ammonium−amino hydrogen bridges which could explain the stability of the tubular phase. Surprisingly, the spectrum of Figure 1c also contained a weak second signal at +47 ppm which grew slowly within several days during which the sample was kept in the rotor. When the sample was dissolved again in 2 M HCl and reprecipitated at pH 10.5 the low-field signal had disappeared but slowly reappeared.

We suspected carbon dioxide as the origin for these spectral changes and therefore exposed the sample of Figure 1c for 24 h to 1 atm of 90% enriched $^{13}CO_2$. As expected, the signal at +47 ppm strongly increased and the remaining amino headgroup signal shifted to −5 ppm (Figure 1d). The incorporation of $^{13}CO_2$ was followed by ^{13}C CPMAS NMR.[12] A few scans (Figure 1e) revealed a single dominant line at 164 ppm, which is typical for carbamates in aqueous solution.[13]

As the signal could stem from both the nonlabeled and the labeled amino groups of **1,** we also measured the $^{13}C-^{15}N$ dipolar coupling which yields directly the corresponding distance. In principle, this information could have been obtained by analysis of the static powder spectra,[15] but we preferred here to use the REDOR technique[6] that employs MAS and has the advantage of high chemical shift resolution and high sensitivity. The results are depicted in Figure 1f. In the REDOR spectrum the signal intensity of ^{13}C attached to ^{15}N is reduced as compared to the echo reference spectrum because of the dipolar $^{13}C-^{15}N$ coupling.[16] A reduction of ≈75% is observed indicating indeed the formation of $^{13}C-^{15}N$ pairs. Assuming that both amino groups react, the signal component arising from the $^{13}C-^{15}N$ pairs is therefore reduced to ≈50% of its original value. With the spinning

(1) Presented at the Symposium on Solid State Reactivity of the American Association of Pharmaceutical Science Meeting, San Francisco, November 16−19, 1998.

(2) (a) Siegfried, M. *Ber. Dtsch. Chem. Ges.* **1906**, *39*, 397. (b) Wright, H. B.; Moore, M. B. *J. Am. Chem. Soc.* **1948**, *70*, 3865.

(3) (a) Henriques, O. M. *Biochem. Z.* **1928**, *200*, 124. (b) Roughton, F. J. W. *Physiol. Rev.* **1935**, *80*, 143. (c) Lilmatin, J. V. *Physical. Rev.* **1997,** *53*, 836.

(4) (a) Caplow, M. *J. Am. Chem. Soc.* **1968**, *90*, 6795. (b) Jensen, A.; Faurholt, C. *Acta Chem. Scand.* **1952**, *6*, 385. (c) Frahn, J. L.; Mills, J. A. *Aust. J. Chem.* **1964**, *17*, 256. (d) Itoh, M. *Chem. Pharm. Bull.* **1972**, *20*, 664. (e) Chen, J. G.; Sandberg, M.; Weber, S. G. *J. Am. Chem. Soc.* **1993**, *115*, 7343. (f) Lemieux, R. U.; Barton, M. A. *Can. J. Chem.* **1971**, *49*, 767. (g) Danckwerts, P. V. *Chem. Eng. Sci.* **1979**, *34*, 443. (h) Crooks, J. E.; Donellan, J. P. *J. Chem. Soc., Perkin Trans. 2* **1989**, 331. (i) Kilmartin, J. V.; Rossi-Bernardi, L. *Nature* **1969**, *222*, 1243. (j) Arnone, A., *Nature,* **1974**, *247*, 143.

(5) (a) Andrew, E. R.; Bradbury, A.; Eades, R. G. *Nature* **1958**, *182*, 1659. (b) Schaefer, J.; Stejskal, E. O.; Buchdahl, R. *Macromolecules* **1975**, *8*, 291. (c) Schaefer, J.; Stejskal, E. O. *J. Am. Chem. Soc.* **1976**, *98*, 1031.

(6) Gullion, T.; Schaefer, J. *J. Magn. Reson.* **1989**, *81*, 196.

(7) Fuhrhop, J.-H.; Spiroski, D.; Boettcher, C. *J. Am. Chem. Soc.* **1993**, *115*, 1600.

(8) Modified synthesis with ^{15}N, starting from 99% enriched $KC^{15}N$.

(9) (a) Benedict, H.; Limbach, H. H.; Wehlan, M.; Fehlhammer, W. P.; Golubev, N. S.; Janoschek, R, *J. Am. Chem. Soc.* In press. (b) López, C.; Lorente, P.; Claramunt, R. M.; Marín, J.; Foces-Foces, C.; Llamas-Saiz, A. L.; Elguero, J.; Limbach, H. H. *Ber. Bunsen-Ges. Phys. Chem.* **1998**, *102*, 414. (c) Braun, J.; Schlabach, M.; Wehrle, B.; Köcher, M.; Vogel, E.; Limbach, H. H. *J. Am. Chem. Soc.* **1994**, *116*, 6593. (d) Braun, J.; Schwesinger, R.; Williams, P. G.; Morimoto, H.; Wemmer, D. E.; Limbach, H. H. *J. Am. Chem. Soc.* **1996**, *118*, 11101.

(10) Reference: solid $^{15}NH_4Cl$. Some authors use neat nitromethane for ^{15}N CPMAS chemical shifts but although these shifts can be converted into solid $^{15}NH_4Cl$ reference by using δCH_3NO_2 + 338.1 ppm (355.3 ppm from CH_3NO_2 to saturated $^{15}NH_4Cl-D_2O$ and −17.2 ppm from saturated $^{15}NH_4Cl-D_2O$ to solid $^{15}NH_4Cl$),[15] they are not directly comparable because they have been calculated by using some approximate relationship between chemical shift references.

(11) (a) Witanowski, M.; Stefaniak, L.; Szymanski, S.; Januszewski, H. *J. Magn. Reson.* **1977**, *28*, 217. (b) Witanowski, M.; Stefaniak, L.; Webb, G. A. Annual Reports on NMR Spectroscopy, 11 B; Academic Press: New York, 1981. (c) Martin, G.; Martin, M. L.; Gouesnard, J. P. *NMR−Basic Principles and Progress*; Springer: Heidelberg, Germany, 1981; Vol. 18, ^{15}N NMR Spectroscopy.

(12) ^{13}C CPMAS spectrum (reference TMS), measured at 5 kHz rotation speed, after a dephasing time of 0.8 ms.

(13) (a) Sherry, A. D.; Malloy, C. R.; Jeffrey, F. M. H.; Chavez, F.; Srere, H. K. *J. Magn. Reson.* **1990,** 89, 391. (b) Wen, N.; Brooker, M. H. *J. Phys. Chem.* **1995**, *99*, 359.

(14) Harris, R. K.; Oliveiri, A. C. *Prog. Nucl. Magn. Reson. Spectrosc.* **1992**, *24*, 435.

(15) Buntkowsky, G.; Sack, I.; Limbach, H. H.; Kling, B.; Fuhrhop, J. H. *J. Phys. Chem. B* **1997**, *101*, 11265.

10.1021/ja981747g CCC: $18.00
Published on Web 05/07/1999

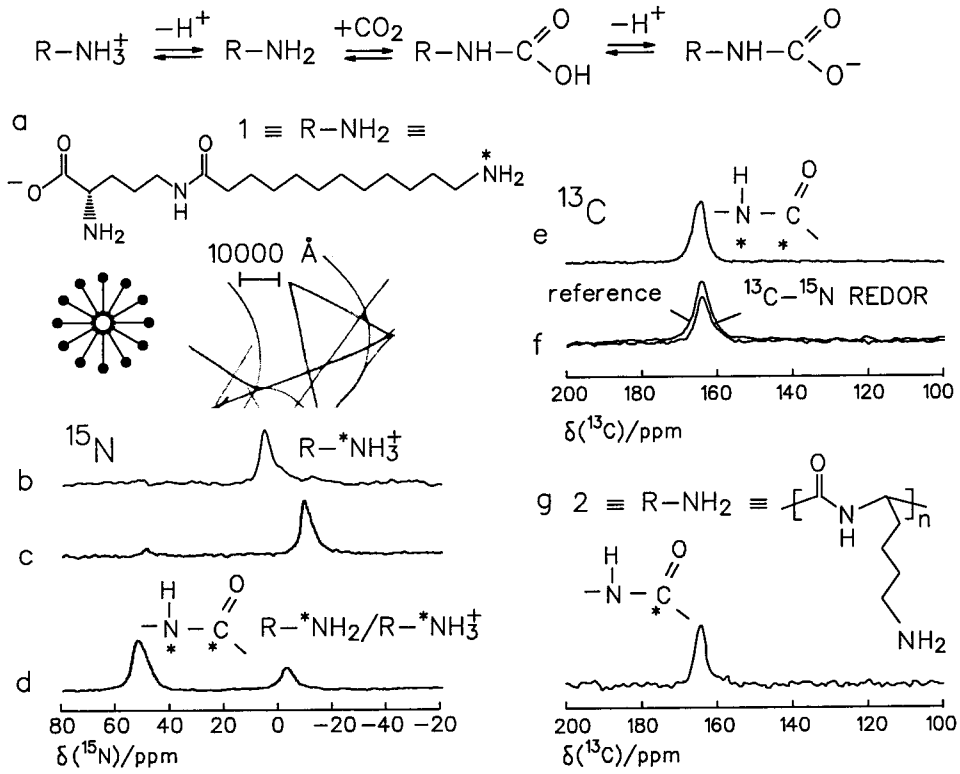

Figure 1. (a) Chemical structure of deprotonated ornithine-amino bolaamphiphile **1**, schematic structure of the monolayered tubular vesicles, and electron micrograph of the fibers formed by the vesicles. (b) ^{15}N CPMAS NMR spectrum of **1** obtained after lyophilization at pH 5. Reference, solid $^{15}NH_4Cl$; 5 kHz spinning speed. (c) ^{15}N CPMAS NMR spectrum of **1**, obtained immediately after precipitation at pH 10.5 and (d) after 24 h of storage under 1 atm of $^{13}CO_2$. (e) ^{13}C CPMAS NMR spectrum corresponding to Figure 1d, 5 kHz spinning speed. Reference, tetramethylsilane; rotational sidebands smaller than about 5%. (f) ^{13}C−^{15}N REDOR (lower trace) and reference spectrum (upper trace), dephasing time 0.8 ms. (g) ^{13}C CPMAS NMR spectrum of poly-l-lysine (SIGMA) obtained from the hydrochloride by adding KOH to a pH of 10.5, exposed 6 days to 1 bar of $^{13}CO_2$ and lyophilized. Asterisks indicate either a ^{15}N or a ^{13}C label. No residual dipolar−quadrupolar ^{13}C−^{14}N splitting was observed.

speed (controlled) of 5 kHz and the dephasing time of 0.8 s (4 rotor cycles) we calculate[6] a dipolar ^{13}C−^{15}N coupling constant of ≈1.2 kHz which is typical for amides,[15] and which corresponds to a ^{13}C···^{15}N distance of about 1.4 Å. The assumption that only the labeled amino group reacts would lead to an incorrect coupling constant of 700 Hz, i.e., an incorrect ^{13}C-^{15}N distance of 1.7 Å.[16] Therefore, we conclude that the nonlabeled amino group has also reacted, which is understandable in view of its lower pK_a value.[17]

To check whether nontubular solids can also react with atmospheric CO_2 we exposed solid poly-l-lysine **2** lyophilized at pH 10.5 for 6 days to 1 atm of $^{13}CO_2$. The resulting ^{13}C CPMAS NMR spectrum (Figure 1g) also showed a dominating carbamate peak around 164 ppm. Finally, we note that similar NMR results have been observed by Schaefer et al.[18] in the case of free amino side chains of a synthetic polymer indicating that this reaction occurs in general.

In conclusion, we have shown by NMR that ammonium/amino groups can bind CO_2 in the dry solid state. Using this method, it should be possible to monitor acidity changes of amino groups of proteins, e.g., during the lyophilization process, a problem of interest in the long-time stability and other properties of theses solids. Such changes can occur because the local dielectric constant is lowered by the water removal leading to an increased acidity of the counterions.[19] Moreover, the method can be used for introducing specific ^{13}C spin labels into biological compounds for NMR purposes.

Acknowledgment. This work was supported by the German Israel Foundation, Jerusalem, the Deutsche Forschungsgemeinschaft, Bonn Bad Godesberg (SFB 448), and the Fonds der Chemischen Industrie, Frankfurt.

JA981747G

(16) The REDOR spectrum was measured on a 7T spectrometer (297.8 MHz ^{1}H frequency), using a sequence of ^{15}N π-pulses, employing an XY-phase cycle,[15] and a spinning speed of 5 kHz, which is high enough to concentrate more than 95% of the integral line intensity in the spinning center band and to suppress residual ^{15}N ^{13}C and ^{14}N ^{13}C dipolar couplings on the ^{13}C line. 128 scans have been accumulated with a repetition time of 3 s. The 90° pulse width was 6.5 μs for all three channels, corresponding to 38 kHz B_1-field in frequency units. CP time was 3 ms. The result after four rotor cycles of REDOR dephasing is shown in the lower trace of Figure 1f.

(17) A major molecular anisotropic motion of the C···N vector such as, for example, a 90° flip or rotation of the −NC vector along an axis perpendicular to the bond direction would lead to a similar reduction of the dipolar coupling. However, there was no such evidence in similar compounds.[15]

(18) Schaeffer, J. Personal communication, 1998.

(19) (a) Golubev, N. S.; Denisov, G. S.; Smirnov, S. N.; Shchepkin, D.; Limbach, H. H. *Z. Phys. Chem.* **1996**, *196*, 73. (b) Ramos, M.; Alkorta, I.; Elguero, J.; Golubev, N. S.; Denisov, G. S.; Benedict, H.; Limbach, H. H. *J. Phys. Chem.* **1997**, *101*, 9791.