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Novel phenomena in the ^{13}C NMR spectra of amino acids

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Summary. ^{13}C NMR integral areas and spin-lattice relaxation times (T_1's) of a series of amino acids were determined at different concentrations. It was found that the spin-lattice relaxation times were markedly reduced in 3 M magnesium chloride resulting in the integral area being proportional to the number of carbon atoms producing each particular signal, with a reliability in excess of 95%. Magnesium chloride is proposed as a ^{13}C NMR relaxation agent for amino acids.

Keywords: Amino acids – Magnesium chloride – ^{13}C NMR relaxation agent – Spin-lattice relaxation time

1. Introduction

NMR technology has been successfully used for the determination of the three-dimensional structure of proteins in solution. Based on two-dimensional NMR spectroscopy, a sequential assignment of peptide backbone protons (H^N and H^{α}) can be outlined. An early assignment example showing a two-dimensional combined NOESY/COSY connectivity diagram for the protein *BPTI* (Bovine Pancreatic Trypsin Inhibitor) in deuterated aqueous solution was outlined in 1981 (Wagner et al., 1981). With the increase in the number of amino acid residues, proton signal overlap in NOESY/COSY connectivity diagrams is marked, making it necessary to introduce a third dimension. ^{15}N and ^{13}C are appropriate for this purpose, but the natural abundances of ^{15}N and ^{13}C are only 0.365% and 1.1% respectively, and the signal strength of ^{15}N and ^{13}C must be enhanced. A possible solution to this is ^{15}N and ^{13}C labeling (Croasman and Carlson, 1994).

This paper extends an earlier observation by us concerning the enhancement of ^{13}C relaxation of amino acids in electrolyte solutions (Tian et al., 2002). Initially, we found a great difference between the ^{13}C NMR spectra of magnesium glycinate and glycine (see Fig. 1): the integral areas of the carbon atom signals of magnesium glycinate are practically identical. By recording the ^{13}C NMR spectra of a series of amino acids paying special attention to integral area, we made a more general observation, which may be useful under some circumstances for the structural determination of proteins.

2. Material and methods

2.1 Chemicals

The amino acids and other chemicals were of analytical grade and were used without further purification.

2.2 Sample preparation for NMR and T_1 determination

Samples were dissolved with the aid of sonication which has the effect of degassing, and the samples were degassed again by sonication for 30 seconds immediately before each determination.

2.3 ^{13}C NMR and spin-lattice relaxation time measurements

All of the ^{13}C NMR spectra were obtained with a Bruker DPX-300 NMR instrument, using an NOE-suppressed inverse gated decoupling pulse sequence with a recycle delay of 8.00 sec and a sweep width of 30120.48 Hz, at $20 \sim 25°C$. For integration, the signal-to-noise ratio of the ^{13}C NMR signals was allowed to exceed 40:1. The integral of the carbon signal with the lowest chemical shift was assigned the arbitrary value of 10.00.

Spin-lattice relaxation times were determined by using an inversion recovery sequence according to the Bruker Avance user's guide. Some key acquisition parameters are relaxation delay (50–200 s), delay list (200 s, 100 s, 50 s, 40 s, 30 s, 20 s, 10 s, 5 s, 4.5 s, 4 s, 3.5 s, 3 s, 2.5 s, 2 s, 1 s, 0.5 s, 0.1 s and 0.01 s), and PL1 (high power level on the f1 channel: 5.70 μs).

3. Results and discussion

The investigation of the effect of magnesium oxide, calcium oxide and sodium hydroxide on the area of the

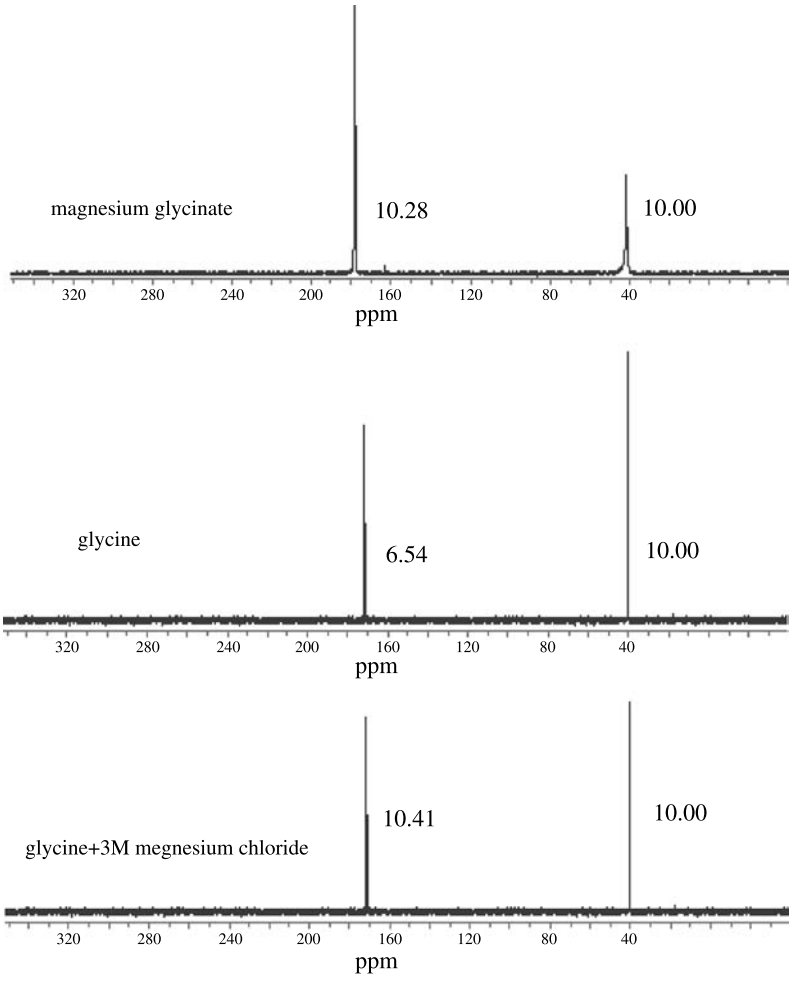


Fig. 1. ^{13}C NMR of magnesium glycinate* (46%) and glycine, without or with 3 M $MgCl_2$. *Magnesium glycinate in Figure 1 was prepared by reacting glycine with excess magnesium oxide. Calcium oxide was used correspondingly to maintain consistency with that of magnesium oxide in Table S1. Considering the low solubilities of magnesium oxide and calcium oxide, reaction time was prolonged

amino acid carbon signals was begun (see Table S1) as a consequence of the observations shown in Fig. 1. The results showed that, generally, when amino acids were converted into their alkali or alkali-earth salts, the integral areas of the carboxyl group ^{13}C NMR signals were increased as compared with the parent amino acid in the following sequence: calcium salts > magnesium salts > sodium salts > parent amino acid. The integral areas of the signals of their salts, however, were not proportional to the number of carbon atoms associated with a particular signal. This will be discussed later.

The pH values of the amino acid solutions in Table S1 were raised by addition of magnesium oxide, calcium oxide or sodium hydroxide. The influence of pH changes on the ^{13}C NMR signal integral areas is exemplified for L-Ala in Table S2. The integral area of the carboxyl carbon signal increased with the increase or decrease of the pH of the L-Ala solution (with a minimum near the pI of L-Ala). Nevertheless, the influence of changing pH on the integral area is not large until the concentration of acid or base is quite high, such as 2.0 M for hydrochloric acid, 15 M for ammonia, and 10 M for sodium hydroxide, with pH values close to 0 or 14.

The influence of metal ions on the integral areas was investigated by adding electrolytes to the solution of L-Ala (Tian et al., 2002). With increasing concentration of electrolyte the integral area of the carboxyl carbon signal relative to the C_α signal area increased, and the integral area of the C_α signal decreased slightly. For NaCl and NH_4Cl, the reliability of the integral areas as a measure of the number of carbon atoms producing each particular signal was about 75% at 5 M concentration, which is close to saturation. On the other hand, when the concentrations of $MgCl_2$ and $CaCl_2$ amounted to 2.9 M and 3 M, respectively, the integral areas of all three L-Ala signals were equal within 95%.

The ^{13}C NMR spectra of a series of amino acids were recorded in 3 M $MgCl_2$ and the results (see Table 1)

Table S1. ^{13}C chemical shifts and integral areas (in parentheses) relative to the highest field signal taken as 10.00, of amino acids and their salts

Amino acid (g)[1]		Additive (g)		pH	Chemical shift (ppm) & integral (in parentheses) relative to the highest-field signal taken as 10.00						
Gly	5.00	Control	0	6.01	171.75 (5.68)	40.42 (10.00)					
		MgO	2.00	9.71	178.50 (8.62)	42.29 (10.00)					
		CaO	2.80	12.31	181.40 (9.04)	43.73 (10.00)					
		NaOH	4.00	13.40	180.57 (5.90)	43.97 (10.00)					
L-Ala	3.40	Control	0	6.00	174.94 (5.16)	49.76 (11.46)	15.43 (10.00)				
		MgO	1.14	9.60	178.44 (6.27)	49.89 (10.46)	17.15 (10.00)				
		CaO	1.60	12.25	183.94 (7.47)	50.48 (10.20)	19.68 (10.00)				
		NaOH	2.36	13.27	183.49 (5.67)	50.58 (11.27)	19.76 (10.00)				
L-Ser	0.50	Control	0	5.97	171.54 (3.90)	59.44 (9.18)	55.68 (10.00)				
		MgO	0.29	9.92	178.42 (5.03)	62.98 (10.83)	56.33 (10.00)				
		CaO	0.40	12.38	179.97 (5.43)	63.75 (8.90)	56.73 (10.00)				
		NaOH	0.57	13.10	179.76 (5.18)	64.04 (9.44)	56.98 (10.00)				
L-Asp	0.20	Control	0	3.04	181.25 (6.24)	179.22 (5.84)	53.19 (12.01)	42.36 (10.00)			
		MgO	0.12	9.90	179.54 (6.64)	179.11 (7.11)	51.71 (9.18)	38.74 (10.00)			
		CaO	0.13	12.47	181.64 (6.91)	179.14 (7.18)	52.70 (10.15)	41.37 (10.00)			
		NaOH	0.19	12.69	181.21 (6.21)	179.18 (6.38)	53.15 (13.26)	42.32 (10.00)			
L-Pro	3.20	Control	0	6.00	173.70 (4.16)	60.38 (10.00)	45.30 (9.39)	28.08 (9.77)	22.88 (10.00)		
		MgO	0.83	9.81	175.12 (4.54)	60.40 (9.48)	45.32 (9.56)	28.38 (9.28)	23.26 (10.00)		
		CaO	1.17	12.26	182.37 (8.81)	60.69 (10.58)	45.36 (10.33)	29.74 (10.41)	24.53 (10.00)		
		NaOH	1.67	13.25	181.61 (6.20)	60.64 (9.62)	45.14 (9.61)	29.85 (9.49)	24.29 (10.00)		
L-Glu	0.20	Control	0	3.27	176.84 (6.91)	173.48 (6.46)	53.66 (9.69)	29.75 (9.05)	25.28 (10.00)		
		MgO	0.12	9.73	180.95 (6.58)	176.74 (6.06)	54.23 (9.61)	32.81 (9.33)	27.74 (10.00)		
		CaO	0.12	12.40	182.43 (6.57)	182.14 (6.31)	55.47 (9.33)	33.56 (9.12)	30.94 (10.00)		
		NaOH	0.17	12.84	182.06 (6.64)	181.92 (6.31)	55.19 (10.36)	33.22 (10.04)	30.96 (10.00)		
L-Lys	1.46	Control	0	10.05	180.35 (6.15)	55.73 (10.12)	38.71 (9.75)	32.22 (9.34)	26.48 (9.52)	20.96 (10.00)	
		MgO	0.30	10.33	181.16 (7.17)	54.78 (10.94)	38.82 (10.51)	32.60 (10.06)	26.89 (10.84)	21.06 (10.00)	
		CaO	0.42	12.39	182.96 (9.06)	55.09 (11.31)	39.64 (10.98)	33.54 (11.09)	30.69 (11.58)	21.45 (10.00)	
		NaOH	0.60	13.04	182.76 (6.71)	55.20 (10.28)	39.69 (10.47)	33.66 (10.41)	30.88 (10.06)	21.45 (10.00)	
L-His	0.90	Control	0	7.82	173.26 (6.07)	135.56 (10.99)	131.28 (7.14)	116.04 (11.01)	54.12 (10.63)	27.44 (10.00)	
		MgO	0.19	9.72	179.42 (7.75)	135.20 (9.89)	132.40 (8.78)	116.53 (9.12)	54.58 (10.16)	29.84 (10.00)	
		CaO	0.27	12.40	181.48 (7.60)	135.13 (10.79)	132.60 (8.19)	117.05 (10.61)	55.22 (9.99)	31.05 (10.00)	
		NaOH	0.38	12.94	181.25 (6.42)	135.38 (10.12)	132.54 (7.13)	117.41 (10.49)	55.32 (10.33)	31.18 (10.00)	
L-Phe	1.00	Control	0	6.23	173.54 (7.01)	134.66 (8.8.10)	128.92 (21.47)	128.66 (21.13)	127.23 (10.77)	55.77 (11.17)	35.99 (10.00)
		MgO	0.19	9.81	179.64 (7.67)	136.61 (9.63)	128.54 (23.25)	127.88 (20.06)	126.06 (10.21)	56.20 (10.18)	38.79 (10.00)
		NaOH	0.37	12.96	181.40 (6.46)	137.35 (8.66)	128.53 (22.47)	127.69 (22.52)	125.74 (10.73)	56.68 (10.23)	39.91 (10.00)

[1] Amino acids were dissolved in 20 ml water (mostly as saturated solutions) and 50% excess magnesium oxide, calcium oxide or sodium hydroxide was added, the mixture was stirred at 50°C for 48 hours to reach equilibrium. The solution was centrifuged and the filtrate was analyzed by ^{13}C NMR

Table S2. Influence of pH on the chemical shifts and integral areas (in parentheses) relative to the highest-field signal taken as 10.00

L-Ala[1] (mol/l)	NaOH (mol/l)	$NH_3 \cdot H_2O$ (mol/l)	HCl (mol/l)	pH	Chemical shift (ppm) & integral area		
1.00	0	0	0	6.86	174.79 (4.40)	49.62 (11.51)	15.24 (10.00)
1.00	6.75E-03	0	0	7.93	174.86 (5.14)	49.64 (11.11)	15.28 (10.00)
1.00	2.70E-02	0	0	8.53	175.02 (5.45)	49.65 (11.08)	15.35 (10.00)
1.00	1.25	0	0	13.49	183.43 (5.83)	50.46 (11.46)	19.47 (10.00)
1.00	2.50	0	0	13.52	183.37 (5.90)	50.37 (11.27)	19.52 (10.00)
1.00	10.00	0	0	>14	183.10 (11.98)	50.04 (10.23)	19.96 (10.00)
2.00	10.00	0	0	>14	182.93 (10.39)	50.07 (9.63)	20.06 (10.00)
1.00	0	0.50	0	9.86	177.09 (4.55)	49.84 (11.07)	16.37 (10.00)
1.00	0	1.00	0	10.18	178.23 (4.77)	49.96 (11.19)	16.96 (10.00)
1.00	0	2.00	0	10.54	179.41 (4.82)	50.06 (11.27)	17.57 (10.00)
1.00	0	15.00	0	>14	180.76 (9.18)	50.27 (11.75)	18.96 (10.00)
1.00	0	0	0.50	2.11	173.39 (8.38)	48.80 (11.09)	14.88 (10.00)
1.00	0	0	1.00	0.87	171.96 (8.14)	48.03 (11.06)	14.50 (10.00)
1.00	0	0	2.00	0.25	171.60 (9.57)	47.99 (10.94)	14.51 (10.00)
1.00	0	0	5.00	0.08	171.29 (10.96)	48.11 (11.75)	14.60 (10.00)[2]
1.00	0	0	–[3]	<0	170.71 (11.57)	48.27 (8.91)	14.53 (10.00)

[1] L-Ala and acids or bases were made up into a volume of 10.00 ml

[2] The peak is split into a quadruplet; the total integral is assigned as 10.00

[3] Concentrated hydrochloric acid solution (36%) was used as solvent instead of water

showed that their integral areas were quite proportional to the number of carbon atoms producing each particular signal with a reliability of over 95%, without special restrictions due to their structure, polarity or concentration. It can be seen in Figure 1 that the intensities of the two ^{13}C signals of glycine are nearly equal in 3 M $MgCl_2$.

We suggest that intermolecular interactions change upon addition of electrolyte, which in turn accelerates the ^{13}C relaxation rate, hence changing the integral areas. Amino acids can be solvated and may form dimers, trimers and higher polymers through intermolecular hydrogen bonding in aqueous solution. It has been reported by Rode (Rode, 1999) that in sodium chloride solutions at concentrations above 3 M, the cation's primary solvation shell becomes 'unsaturated'. This means that the average coordination number of six water molecules in the first shell can no longer be realized for sodium ions. A similar ''water defect'' occurs in other electrolytes at high concentrations, favoring the aggregation of molecules and enhancing their structural rigidity. It is well known that the relaxation rate increases (T_1 decreases) with enhancement of molecular rigidity and aggregation (Breitmaier and Voelter, 1978).

Complex formation between amino acids and magnesium ions is also important for the increase of the ^{13}C relaxation rate. Amino acids can form complexes with magnesium and calcium, though the stability of these complexes is not high (Greenstein and Wintz, 1961). In concentrated electrolyte, amino acids are more prone to enter the inner solvation sphere of the metal ions and, as a result, they are ''anchored'' and molecular tumbling is slowed down, molecular rigidity is increased and molecular bulk also increases. All of these factors contribute to increase the ^{13}C relaxation rate (Levy et al., 1973, 1974). Ammonium and sodium ions cannot form *cyclo*-complexes with amino acids in the same way as magnesium and calcium ions can (Tian et al., 2002), and as a result the integral areas of the L-Ala signals are not equal even in 5 M NaCl or NH_4Cl.

Thus, both the ''water defect'' and complex formation in concentrated $MgCl_2$ and $CaCl_2$ are indispensable for acceleration of the ^{13}C relaxation rate as can be seen by the spin-lattice relaxation times (T_1) of amino acids in the absence or presence of 3 M $MgCl_2$ (Table 2). At this concentration, both $MgCl_2$ and $CaCl_2$ cause reduced T_1 values for all the carbon nuclei of amino acids in comparison with those observed in the absence of these electrolytes. The T_1 value*s* of all the carbons are reduced below 8.00 s, which is the recycle delay we set in the ^{13}C NMR experiments. Thus, all the carbons can relax to their equilibrium distribution between successive pulses, and the integral area of the carbon atom signals becomes proportional to the number of carbon atoms that produce each particular signal.

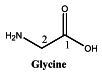
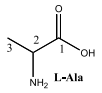
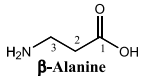
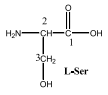
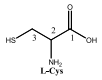
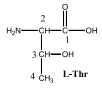
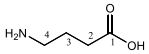
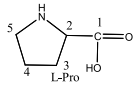
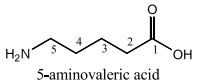
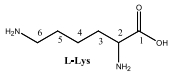
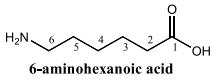
With this in mind, let us reconsider the sequence of the carboxyl signal areas in Table S1: calcium salts >

Table 1. Influence of 3 M $MgCl_2$ on the chemical shifts and integrals of amino acid ^{13}C NMR signals

Amino acid (AA)	[AA] (mol/l)	[$MgCl_2$] (mol/l)	PH[4]	Chemical shift (ppm) & integral (in parentheses) relative to the highest-field signal taken as 10.00					
Gly	1.00	3	6.01	171.39 (10.41)	40.69 (10.00)				
Iminodiacetic acid	0.30	3	0.56	165.58 (10.81)	46.98 (10.00)				
DKP[1]	0.08	3	3.82	165.58 (10.81)	46.98 (10.00)				
L-Ala	1.00	3	5.42	177.53 (9.93)	35.79 (9.92)	32.22 (10.00)			
EDTA[2]	0.46	3	–	169.60 (22.13)	57.32 (22.51)	50.92 (10.00)			
L-Cys	1.00	3	3.43	171.16 (10.52)	54.85 (10.52)	23.90 (10.00)			
L-Ala	0.25	3	4.37	174.40 (10.74)	49.54 (9.49)	15.21 (10.00)			
L-Ala	0.50	3	4.45	174.54 (10.74)	49.67 (10.03)	15.35 (10.00)			
L-Ala	1.00	3	4.40	174.53 (10.78)	49.66 (9.92)	15.37 (10.00)			
L-Ser	0.05	3	4.05	171.18 (10.93)	59.98 (11.25)	55.01 (10.00)			
L-Ser	0.10	3	3.94	171.11 (10.65)	58.91 (9.75)	54.94 (10.00)			
L-Ser	0.33	3	3.93	171.33 (9.85)	59.11 (9.83)	55.14 (10.00)			
L-Ser	1.00	3	3.59	171.16 (10.52)	54.85 (10.52)	23.90 (10.00)			
L-Thr	0.05	3	4.26	171.40 (10.73)	64.71 (11.05)	58.63 (10.68)	18.29 (10.00)		
L-Thr	0.10	3	3.88	171.42 (9.72)	64.73 (10.18)	58.65 (9.51)	18.32 (10.00)		
L-Thr	0.15	3	3.95	171.43 (9.86)	64.75 (9.99)	58.67 (9.46))	18.34 (10.00)		
L-Thr	0.20	3	3.81	171.43 (10.65)	64.74 (10.34)	58.66 (10.17)	18.34 (10.00)		
L-Thr	1.00	3	3.55	171.59 (10.10)	58.86 (9.79)	64.92 (9.81)	18.57 (10.00)		
EDTP[3]	0.37	3	1.75	174.92 (21.83)	28.44 (21.37)	46.58 (10.00)	49.78 (20.61)		
4-Aminobutyric acid	1.00	3	5.84	180.84 (10.14)	22.39 (10.00)	33.32 (9.95)	38.94 (9.60)		
***N*-acetylglycine**	0.20	3	–	174.45 (8.55)	41.17 (9.45)	173.26 (8.59)	21.77 (10.00)		
Glycinylglycine	0.12	3	4.70	175.61 (9.90)	42.63 (9.54)	166.00 (10.36)	40.00 (10.00)		
L-Asp	0.03	3	1.26	173.40 (9.82)	171.58 (10.61)	49.68 (10.27)	33.60 (10.00)		
L-Glu	0.05	3	1.51	176.25 (12.74)	52.87 (12.71)	24.21 (10.00)	29.23 (9.88)	172.55 (11.34)	
L-Pro	1.00	3	4.21	173.49 (9.99)	61.15 (10.07)	27.77 (10.14)	22.80 (10.00)	45.55 (9.86)	
5-Aminovaleric acid	1.00	3	5.64	183.01 (9.86)	26.38 (10.01)	22.17 (10.00)	36.35 (9.90)	39.62 (9.98)	
L-Lys	1.00	3	7.58	178.74 (9.69)	53.43 (10.20)	30.62 (9.77)	20.94 (10.00)	25.46 (10.17)	38.79 (9.71)
L-Lys-HCl	1.00	3	3.84	173.32 (10.44)	53.55 (10.11)	28.53 (10.05)	20.30 (10.00)	25.25 (10.00)	38.54 (9.91)
L-His	0.20	3	4.3	174.19 (9.03)	53.13 (9.85)	26.37 (10.00)	128.37 (9.73)	117.78 (9.33)	135.09 (9.77)
L-His-HCl	0.20	3	3.0	171.38 (11.24)	52.64 (10.24)	24.51 (10.00)	125.81 (12.25)	117.07 (11.51)	133.05 (10.06)
6-Aminohexanoic acid	1.00	3	6.34	182.96 (10.04)	36.09 (9.81)	24.05 (9.41)	24.45 (10.06)	25.60 (10.09)	39.03 (10.00)

[1] *DKP*, 2,5-diketopiperazine
[2] *EDTA*, ethylenediaminotetraacetic acid
[3] *EDTP*, ethylenediaminotetrapropanoic acid
[4] pH of 3 M $MgCl_2$ is 2.96

Table 2. Spin-lattice relaxation times (T_1) of some amino acids in the presence or absence of 3 M $MgCl_2$

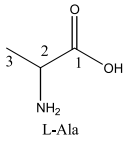
Amino acid	[AA] mol/l	[$MgCl_2$] mol/l	T_1(s)					
			C_1	C_2	C_3	C_4	C_5	C_6
 Glycine	1.00	3	7.941	0.710				
		0	34.966	5.403				
 L-Ala	1.00	3	5.958	1.165	1.286			
		0	31.018	4.023	2.511			
 β-Alanine	1.00	3	5.213	0.412	0.884			
		0	26.646	4.869	3.894			
 L-Ser	1.00	3	6.858	0.957	0.591			
		0	28.117	2.926	1.522			
 L-Cys	1.00	3	8.779	0.938	0.546			
		0	31.066	2.900	2.059			
 L-Thr	1.00	3	4.488	0.531	0.654	1.360		
		0	10.203	1.874	3.877	1.670		
 4-aminobutyric acid	1.00	3	7.984	0.954	0.814	1.180		
		0	20.077	2.465	2.913	2.369		
 L-Pro	1.00	3	8.595	1.392	0.953	1.845	1.442	
		0	37.775	6.139	4.032	4.826	3.400	
 5-aminovaleric acid	1.00	3	7.295	0.737	0.708	1.163	1.119	
		0	27.705	2.244	1.742	2.461	2.620	
 L-Lys	0.50	3	4.789	0.421	0.261	0.385	0.561	0.704
	1.00	0	3.066	1.782	1.189	1.285	2.110	2.376
 6-aminohexanoic acid	1.00	3	4.609	0.552	0.616	0.697	0.821	0.952
		0	26.013	1.790	1.747	1.752	1.644	1.729

* Normally, ^{13}C T_1 data would not be reported to the precision given in Tables 2 and 3. The T_1's in Tables 2 and 3 are of particularly high accuracy, aided by the high concentrations of amino acids and by persistent attention to experimental details

magnesium salts > sodium salts > parent amino acids. Under the conditions given in Table S1, the concentration of metal ion is much lower than 3 M, leading to a small or negligible ''water defect''. Thus, the integral areas for the different ^{13}C signals are not equal. For the parent amino acid solutions, their pH equals pI and no complex formation occurs, so the integral area of the carboxyl signal in the parent amino acid is the smallest in the abovementioned sequence. As the sodium salts cannot form *cyclo*-complexes in the same way as the magnesium and calcium salts can, the integral areas of their carboxyl signals are smaller even though the pH of the sodium salt solutions is higher. The difference between calcium and magnesium salts is partly due to the influence of pH.

The abilities of $CaCl_2$ and $MgCl_2$ to reduce the T_1's of amino acids are quite different. $CaCl_2$ is not as effective as $MgCl_2$; it is not until 4 M concentration of $CaCl_2$ is reached that the T_1's of L-Ala, particularly of the carboxyl carbon nucleus, become similar to those in 3 M $MgCl_2$ (see Table 3). This difference arises mainly from the relative stabilities of the complexes of calcium and magnesium ions with amino acids. For the complexes of Ca (II)

Table 3. Spin-lattice relaxation times of L-Ala in the presence of different concentrations of $MgCl_2$ and $CaCl_2$

L-Ala[1]	$[MgCl_2]$ mol/l	$[CaCl_2]$ mol/l	T_1(s)		
			C_1	C_2	C_3
 L-Ala	0	0	28.036	4.023	2.511
	1	0	16.470	2.607	1.778
	2	0	10.753	1.715	1.429
	3	0	5.96	1.165	1.286
	4	0	3.279	0.489	0.855
	0	1	17.380	3.006	2.100
	0	2	11.088	1.947	1.972
	0	3	7.901	1.061	1.421
	0	4	5.732	0.798	1.017

[1] The concentration of L-Ala was 1.00 mol/l

and Mg (II) with Gly, the stability constants as $\log^{Ks}$ are 1.4 and 3.4 respectively, and with L-Ala they are 1.2 and 2.0 respectively (Greenstein, 1961). We therefore have reasons to prefer $MgCl_2$ as a relaxation agent for amino acids over $CaCl_2$. Though the high acid and base concentrations in Table S2 are also effective to accelerate the ^{13}C relaxation rate, these media may be too harsh for certain studies.

The ^{13}C NMR signal integrals of *N*-acetylglycine and glycinylglycine (in boldface in Table 1) are also approximately equal within 85% and 98% respectively. This means that the relaxation rates of the carbon nuclei involved in peptide bonds can also be accelerated in 3 M $MgCl_2$.

4. Conclusion

The ^{13}C NMR signals integral areas of amino acids are increased when they are converted into alkali or alkali-earth salts. Furthermore, these integral areas show good proportionality to the number of carbon atoms that produce each particular signal with reliability in excess of 95% in 3 M $MgCl_2$ as a result of the reduction in T_1's for the ^{13}C nuclei. $MgCl_2$ is proposed as an efficient ^{13}C NMR relaxation agent for amino acids.

Acknowledgements

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Supporting information available: Chemical shifts and integral areas of amino acids and their salts (Table S1). The influence of pH on the chemical shifts and integral areas of L-Ala ^{13}C NMR signals (Table S2).

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