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Magnesium chloride: an efficient ^{13}C NMR relaxation agent for amino acids and some carboxylic acids

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Abstract

A series of amino acids and carboxylic acids were determined by ^{13}C NMR spectroscopy. The results showed that addition of 3 M $MgCl_2$, led to shortening of relaxation time and ^{13}C NMR integral area of samples was well proportional to the number of carbon atoms with reliability more than 95%. So $MgCl_2$ is proposed as an efficient relaxation agent for analysis of amino acids and some carboxylic acids.

Keywords: Amino acids; Carboxylic acids; Magnesium chloride; ^{13}C NMR spectroscopy; ^{13}C NMR relaxation agent

1. Introduction

Integration reliability of NMR signals is important for quantitative NMR analysis. Peak area of the signals in CW spectra is generally proportional to the number of protons coming into resonance at the frequency of the signals. FT spectra are not quite straightforward. Integral area is reliable with properly taken ^{1}H NMR because all the hydrogen nuclei can relax to their equilibrium distribution between successive pulses. But it is not the case with ^{13}C NMR spectra because the relaxation rate of carbon atoms directly bonded to hydrogen atoms is much higher than that of carbon atoms without being hydrogen bonded. Integral area cannot therefore be used in ^{13}C NMR spectroscopy in the way that it can in ^{1}H NMR spectroscopy, and some peaks are so weak, (carbonyl groups are notorious in this respect) that they do not even appear in the spectrum [1]. Integral area is notoriously disproportional to the number of carbon atoms in ^{13}C NMR spectra with proton broad band decoupling because of NOE effect and relaxation rate difference of all the carbon atoms. A relaxation agent is necessary for quantitative ^{13}C NMR besides using NOE-depressed inverse gated decoupling with sufficiently long recycle delay. It is possible to increase the intensity of weak signals by supplying a powerful magnetic influence, such as a paramagnetic salt, to speed up the relaxation. Chromium acetylacetonate, which is the mostly used relaxation agent in quantitative ^{13}C NMR analysis [2–4], is expensive. It must be pointed out that the above mentioned is applied only to liquid-state quantitative ^{13}C NMR analysis. Several methods have been developed for solid-state quantitative ^{13}C NMR analysis [5–8].

It was found in our previous work that the ^{13}C NMR spectra of magnesium glycinate and glycine are different remarkably; especially the integral area of magnesium glycinate is well proportional to the number of carbon atoms being detected [9]. A more impressive phenomenon was found in that some electrolytes have interesting effects on the ^{13}C NMR integral area of L-Ala when we try to elucidate the mechanism exemplified with it. The influence of electrolytes on the ^{13}C NMR integral area of amino acids and carboxylic acids and its mechanism are investigated in this paper.

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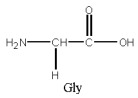
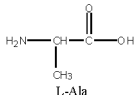
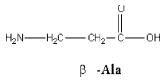
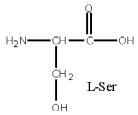
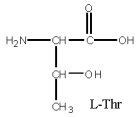
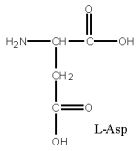
2. Results and discussion

The influence of electrolyte concentration on the ^{13}C NMR integral area of L-Ala is first investigated and the results are listed in Table 1. The integral area of the carbon in the carboxyl group is enhanced with the increase of electrolyte concentration. When the concentration of $MgCl_2$ amounts to 2.90 M, or that of $CaCl_2$

Table 1
Influence of electrolytes on L-alanine's chemical shift and integral area

[L-Ala] (mol/l)	[$MgCl_2$] (mol/l)	[$CaCl_2$] (mol/l)	[NaCl] (mol/l)	[NH_4Cl] (mol/l)	pH	Chemical shift (ppm) and integral area		
1.00	0	0	0	0	6.86	174.79 (4.40)	49.62 (11.51)	15.24 (10.00)
1.00	0.50	0	0	0	5.38	174.26 (6.13)	49.03 (11.01)	14.68 (10.00)
1.00	1.00	0	0	0	5.08	174.23 (7.01)	49.01 (10.68)	14.69 (10.00)
1.00	1.56	0	0	0	5.02	174.24 (9.45)	49.05 (10.53)	14.70 (10.00)
1.00	2.14	0	0	0	4.32	174.08 (9.33)	19.01 (10.07)	14.72 (10.00)
1.00	2.90	0	0	0	3.90	174.20 (9.85)	49.05 (9.87)	14.76 (10.00)
1.00	0	1.02	0	0	5.62	175.08 (7.26)	49.70 (10.86)	15.40 (10.00)
1.00	0	2.01	0	0	5.21	174.99 (8.38)	49.66 (10.14)	15.41 (10.00)
1.00	0	3.07	0	0	4.72	174.86 (9.97)	49.71 (10.47)	15.50 (10.00)
1.00	0	4.05	0	0	4.22	174.72 (10.42)	49.80 (9.82)	15.58 (10.00)
1.00	0	0	0.50	0	6.80	174.95 (4.79)	49.75 (11.80)	15.40 (10.00)
1.00	0	0	1.00	0	6.54	174.95 (4.98)	49.74 (11.52)	15.42 (10.00)
1.00	0	0	2.50	0	6.20	174.93 (5.92)	49.72 (11.25)	15.48 (10.00)
1.00	0	0	5.00	0	5.94	174.91 (7.66)	49.73 (10.24)	15.61 (10.00)
1.00	0	0	0	0.50	6.13	174.81 (5.85)	49.62 (11.46)	15.27 (10.00)
1.00	0	0	0	1.00	6.01	174.81 (6.40)	49.59 (11.86)	15.28 (10.00)
1.00	0	0	0	2.50	5.84	174.79 (7.11)	49.59 (11.54)	15.34 (10.00)
1.00	0	0	0	5.00	5.62	174.73 (7.14)	49.58 (10.72)	15.44 (10.00)

Table 2
Influence of 3 M $MgCl_2$ medium on ^{13}C NMR integral area of amino acids

AA	[AA] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area				Pulse
 Gly	556.66	3	6.01	171.40 (10.41)	41.04 (10.00)			zgig[a]
 L-Ala	1000.00	**3**	4.40	174.55 (5.35)	49.77 (9.69)	15.46 (10.00)		Zgdc[b]
	1000.00		4.40	174.53 (10.78)	49.66 (9.92)	15.37 (10.00)		Zgig
 β -Ala	1000.00	3	5.42	177.53 (9.93)	35.79 (9.92)	32.22 (10.00)		Zgig
 L-Ser	327.90	3	3.93	171.31 (9.85)	59.10 (9.83)	55.13 (10.00)		Zgig
 L-Thr	200.60	3	3.81	171.43 (10.65)	64.74 (10.34)	58.66 (10.17)	18.34 (10.00)	zgig
 L-Asp	32.68	3	1.26	173.40 (9.82)	171.58 (10.61)	49.68 (10.27)	33.60 (10.00)	zgig

[a] Zgig: acquisition pulse is 1D inverse gated decoupling pulse.
[b] Zgdc: acquisition pulse is 1D sequence with decoupling pulse.

Table 3
Influence of 3 M $MgCl_2$ medium on ^{13}C NMR integral area of amino acids

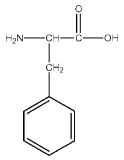
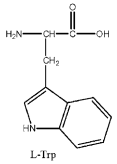
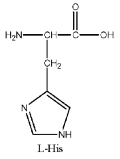
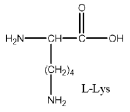
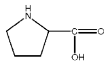
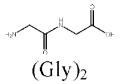
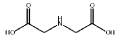
Sub	[Sub] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area						
 L-Phe	Sat.[a]	0	6.23	174.08 (6.94)	135.25 (8.65)	129.49 (22.25)	129.22 (22.08)	127.79 (11.21)	56.34 (11.19)	36.56 (10.00)
	16.3	3	4.75	172.73 (12.41)	133.47 (16.69)	128.39 (34.29)	128.14 (33.07)	126.76 (17.21)	54.96 (13.81)	34.91 (10.00)
 L-Trp	40.00	3	4.07	173.19 (11.59)	134.93 (12.30)	125.32 (12.66)	124.30 (12.11)	121.09 (11.33)	118.40 (12.05)	117.44 (11.15)
				111.07 (11.49)	105.84 (11.90)	53.94 (9.92)	24.98 (10.00)			
 L-His	200.60	3	6.32	174.19 (9.03)	135.09 (9.77)	128.37 (9.73)	117.78 (9.33)	53.13 (9.85)	26.37 (10.00)	
L-His-HCl	169.77	3	3.05	171.73 (11.24)	133.05 (10.06)	125.81 (12.25)	117.07 (11.51)	52.64 (10.24)	13.89 (10.00)	
 L-Lys	501.27	3	7.80	178.74 (9.69)	53.43 (10.20)	38.79 (9.71)	30.62 (9.77)	25.46 (10.17)	20.94 (10.00)	
	501.27	3	7.80	178.74 (9.69)	53.43 (10.20)	38.79 (9.71)	30.62 (9.77)	25.46 (10.17)	20.94 (10.00)	
L-Lys-HCl	500.20	0	7.35	173.85 (7.18)	53.80 (10.57)	38.49 (10.17)	29.13 (9.92)	25.67 (10.30)	20.57 (10.00)	
 L-Pro	503.97	3	6.05	173.55 (10.47)	53.69 (10.22)	38.68 (10.08)	28.69 (9.99)	25.40 (10.20)	20.43 (10.00)	
	203.90	3	4.44	173.56 (9.19)	60.13 (9.86)	45.54 (9.75)	27.74 (9.95)	22.77 (10.00)		
 $(Gly)_2$	115.91	3	4.70	175.61 (9.90)	166.00 (10.36)	42.63 (9.54)	40.00 (10.00)			
 Iminodiacetic acid	297.15	3	0.56	165.58 (10.81)	46.98 (10.00)					

Table 3 (*continued*)

Sub	[Sub] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area				
L-Glu	50.00	3	1.51	176.25 (12.74)	172.55 (11.34)	52.87 (12.71)	29.23 (9.88)	24.21 (10.00)
	24.65	0	4.54	170.55 (6.55)	148.81 (8.23)	130.82 (20.00)	118.85 (5.59)	115.11 (20.53)
p-amino benzoic acid	26.98	3	2.61	171.42 (9.25)	140.88 (10.51)	130.27 (19.23)	126.30 (9.25)	118.28 (20.00)

[a] The solubility of L-Phe is 3 g/100 ml H_2O at 25 °C.

amounts to 3 M, the integral area of all the carbons in L-Ala is well proportional to the number of carbon atoms; the reliability is more than 95%. But the integral area of L-Ala is proportional to the number of carbon atoms only with reliability of about 75%, even in 5.00 M NaCl or NH_4Cl solution which is close to their saturated concentration.

We used 3 M $MgCl_2$ solution as solvent to make series of amino acid and carboxylic acid solutions and then carried out ^{13}C NMR analysis. The results are listed in Tables 2–5.

It is a general phenomenon for α-amino acids as shown in Tables 2 and 3 that their ^{13}C NMR integral area is well proportional to the number of carbon atoms in 3 M $MgCl_2$ medium, the reliability is more than 95%. The integral area of the carbon in carboxyl group is increased outstandingly in this medium. No significant difference exists for amino acids other than α-amino acids, such as β-Alanine, glycinylglycine, and *p*-aminobenzoic acid. Additionally, the integral area of carbons in both aryl group and carboxyl group in amino acids such as L-Phe, L-Trp, and L-His is increased, even exceeding that in methylene group.

It must be stressed that the phenomenon in this medium has its stand only by using the NOE-depressed inverse gated decoupling pulse. The integral area of L-Ala is disproportional to the number of carbon atoms even in 3 M $MgCl_2$ medium as shown in Table 2 if decoupling pulse is used, with which NOE-effect is not depressed.

It is clear that the structure of carboxylic acids is essential to integral area increase in 3 M $MgCl_2$ medium (Tables 4 and 5) and it is not enough only with carboxyl group. As to aliphatic acid and its α-chlorinated derivatives, the integral area of the carbon in carboxyl group is increased slightly. But the integral area of some carboxylic acids with carbonyl group, hydroxyl group, and carboxyl group at α or β position is indeed proportional to the number of carbon atoms in 3 M $MgCl_2$ medium. Thus, by summarizing the structure of samples listed in Tables 2, 3, and 5, we got a general structure, as shown in Chart 1. Once amino groups and carboxyl groups coexist in a molecule, integral area being proportional to the number of carbon atoms with reliability more than 95%. The amino groups are all effective, no matter what position they localize and whether they are normal, secondary or tertiary amino groups.

We do have reasons to believe that relaxation rate of the carbons in amino acids and those carboxylic acids must be accelerated in 3 M $MgCl_2$ medium. This is because a reduction in spin–lattice relaxation time (T_1) of all the carbons in some α-amino acids is produced in 3 M $MgCl_2$ medium, as shown in Table 6. T_1 values, especially those of carbons in carboxyl groups, are almost all reduced below 8.00 s. The relaxation delay in our experiment is set to 8.00 s so that all the carbons can

Table 4
Influence of 3 M $MgCl_2$ medium on ^{13}C NMR integral area of carboxylic acids

Acid	[Acid] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area	
CH_3COOH	556.66	3	0.54	176.45 (7.45)	20.60 (10.00)
CH_3COOH	1050	0	0.70	178.05 (5.11)	20.11 (10.00)
$ClCH_2COOH$	554.49	3	<0	171.30 (8.48)	41.10 (10.00)
$Cl_2CHCOOH$	563.09	3	<0	167.26 (6.41)	64.24 (10.00)
Cl_3CCOOH	—[a]	3	<0	164.96 (14.85)	91.65 (10.00)
Cl_3CCOOH	440.85	0	<0	165.69 (19.49)	93.59 (10.00)

[a] 4.42 mmol trichloroacetic acid, the solubility of which in water is 1600 g/l (20), cannot dissolve in 10 ml 3 M $MgCl_2$; after 24 h equilibration, upper layer was ^{13}C NMR analyzed.

Table 5
Influence of 3 M $MgCl_2$ medium on ^{13}C NMR integral area of carboxylic acids

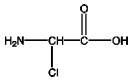
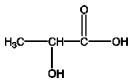
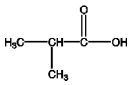
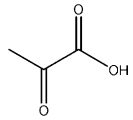
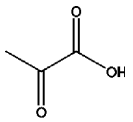
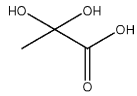
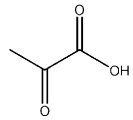
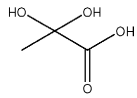
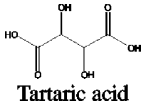
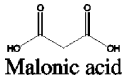
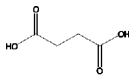
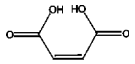
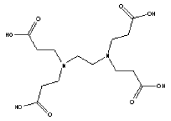
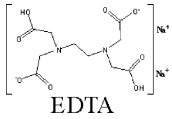
Acid	[Acid] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area			
	499.40[a]	3	<0	173.54 (7.83)	52.65 (11.41)	20.41 (10.00)	
	1321.61	3	<0	177.88 (9.14)	65.77 (9.98)	18.74 (10.00)	
	998.98	3	1.18	181.56 (3.81)	32.41 (5.70)	17.24 (10.00)	
			<0	196.68 (9.31)	162.12 (9.21)	25.76 (9.91)	
	1037.36	3					
 Pyruvic acid				174.13 (9.06)	92.50 (9.74)	25.35 (10.00)	
			<0	197.51 (2.77)	163.84 (2.79)	25.06 (5.05)	
	1017.71	0					
				174.29 (5.05)	92.69 (6.21)	24.38 (10.00)	
 Citric aicd	500.00	3	<0	175.58 (10.09)	172.36 (20.99)	72.31 (10.97)	42.26 (20.00)
 Tartaric acid	500.70	3	<0	173.38 (10.37)	70.73 (10.00)		
 Malonic acid	505.29	3	<0	170.30 (20.76)	39.98 (10.00)		
	308.60	0	1.42	170.48 (10.26)	40.21 (10.00)		

Table 5 (*continued*)

Acid	[Acid] (mmol/l)	[$MgCl_2$] (mol/l)	pH	Chemical shift (ppm) and integral area			
 Succinic acid	120.25	3	0.91	176.20 (9.12)	28.07 (10.00)		
 Maleic acid	502.54	3	<0	168.66 (8.86)	130.28 (10.00)		
	503.92	0	1.30	168.96 (4.42)	130.29 (10.00)		
 EDTP	36.61	3	1.75	174.92 (21.83)	49.78 (20.61)	46.58 (10.00)	28.44 (21.37)
 EDTA	45.94	3	—	169.60 (22.13)	57.32 (22.51)	50.92 (10.00)	

[a] 4.994 mmol substrate cannot be dissolved in 10 ml 3 M $MgCl_2$ solution; after 48 h equilibration, upper layer was ^{13}C NMR analyzed.

relax to their equilibrium distribution between successive pulses. So it certainly can be concluded that the T_1 values of all the carbons in the structure shown in Chart 1 are reduced in 3 M $MgCl_2$ medium.

But what is the mechanism that the T_1 values of all the carbons in the structure shown in Chart 1 are reduced in the 3 M $MgCl_2$ medium? Two key factors besides molecular structure of samples are coordinating ability of metal ion and electrolyte solution with high concentration, which change molecular interaction.

Magnesium ion and calcium ion can form complex with α-amino acids in aqueous solution as shown in Chart 2 and the latter one is more favorable. Rigidity of molecular structure increases with complex formation and compounds with the structure shown in Chart 1 can also form complex with magnesium ion and calcium ion. On the contrary, carboxylic acids in Table 5 cannot form the latter complex shown in Chart 2 readily in 3 M $MgCl_2$ medium so that the integral area of the carbons in carboxyl groups increases only in little degree.

It is not enough to explain the mechanism only with the complex formation shown in Chart 2. Electrolyte solution with high concentration is also an important factor. It has been reported by Rode [11] that: in NaCl solution with concentration above 3 M, the cation's primary solvation shell becomes 'unsaturated'. This means that the average coordination number of 6 water molecules in the first shell can no longer be realized for sodium ions. Similar "water-insufficient" state exists in 3 M $MgCl_2$ medium, which facilitates both intermolecular hydrogen bond formation and complex formation. The pH of solution decreases with addition of electrolyte (seen Table 1) as a result of carboxyl group coming into the first shell of metal ion. Molecular structure rigidity and molecular aggregation of amino acids and carboxylic acids are improved with both intermolecular hydrogen bond formation and complex formation. And it has been known that relaxation rate increases with enhancement of molecular structure rigidity and molecular aggregation [10,12]. Intermolecular hydrogen bond formation and complex formation are both indispensable to decrease T_1 values in electrolyte solution with high concentration. One proof is that the integral area of L-Ala is disproportional to the number of carbon atoms, even in nearly saturated solution of NaCl and NH_4Cl (Table 1) for the weaker coordinating ability of sodium ion and ammonium ion compared with that of magnesium ion and calcium ion. It is carboxyl group that is directly involved in hydrogen bond formation and complex formation, so the T_1 value of its carbon is reduced more markedly (see T_1 value of Gly, L-Ala, and β-Ala in Table 6).

We prefer $MgCl_2$ as a ^{13}C NMR relaxation agent for amino acids and carboxylic acids with structure shown in Chart 1 to $CaCl_2$ because the solubility of the complex between dicarboxylic acid and calcium ion is not good enough. The phenomenon we observed is important for quantitative ^{13}C NMR analysis. $MgCl_2$ is simple but with novel effects and it is easy to control and recover samples.

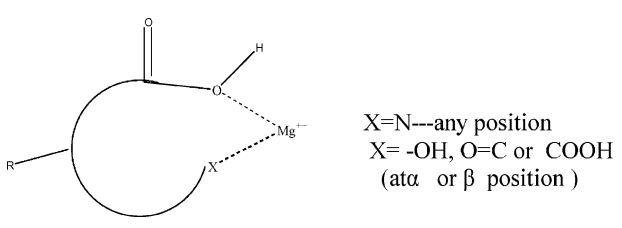
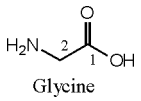
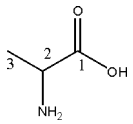
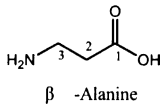
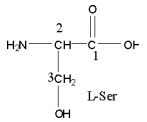
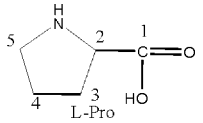
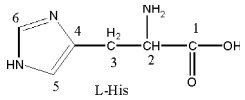
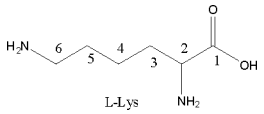


Chart 1. The indispensible structure in 3 M $MgCl_2$ medium.

Table 6
T_1 values of some amino acids in 3 M $MgCl_2$ medium

Amino acid	[AA] (mol/l)	[$MgCl_2$] (mol/l)	T_1 (s)					
			C1	C2	C3	C4	C5	C6
 Glycine	0.557	3	8.671	0.850				
	1.00	0	34.966	5.403				
	1.00	3	5.96	1.165	1.286			
	1.00	0	31.018	4.023	2.511			
 β -Alanine	1.00	3	4.830	0.757	0.860			
	1.00	0	26.646	4.869	3.894			
 L-Ser	0.328	3	6.058	0.503	0.966			
 L-Pro	1.00	3	8.595	1.392	0.953	1.845	1.442	
 L-His	0.201	3	3.011	0.468	0.286	3.504	0.724	0.492
 L-Lys	1.00	3	4.789	0.421	0.261	0.385	0.561	0.704

3. Conclusion

The ^{13}C NMR integral area of compounds with the structure in Chart 1 is proportional to the number of carbon atoms in 3 M $MgCl_2$ medium by using NOE-depressed inverse gated decoupling pulse with reliability more than 95%. T_1 values of all the carbons in those molecules are reduced remarkably in this medium. Electrolyte solution with high concentration, complex formation, and molecular structure are essential factors for the mechanism of this phenomenon. $MgCl_2$ is proposed as a ^{13}C NMR relaxation agent for compounds with the structure in Chart 1, which is useful for their quantitative liquid-state ^{13}C NMR analysis.

4. Experimental

Chemicals. The amino acids and carboxylic acids used in experiments are all of analytical grade purity and used directly without further purification.

General methods. All of the ^{13}C NMR spectra were obtained with a Bruker DPX-300 NMR instrument, using NOE-suppressed inverse gated decoupling with a

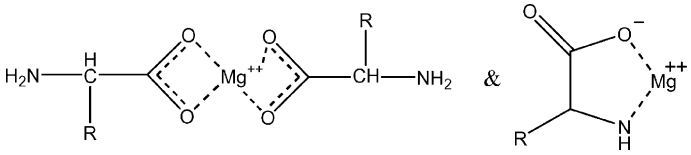


Chart 2. Complex between amino acids and Mg^{++}.

recycle delay of 8.00 s and a sweep width of 30120.48 Hz experiment temperature is 20–25 °C. For integration, the signal-to-noise ratio of the ^{13}C NMR signals is more than 40:1 and integral area of the carbon with the smallest chemical shift is calibrated as 10.00.

T_1 values were determined by using inversion recovery according to Bruker advance user's guide. Some key acquisition parameters are relaxation delay which is 50–200 s, delay list (what we used is: 200, 100, 50, 40, 30, 20, 10, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1, 0.5, 0.1, and 0.01 s), and PL1 (high power level on f1 channel) which is 5.70 μs.

Acknowledgments

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