

# VIBRATIONAL ANALYSIS OF AMINO ACIDS: CYSTEINE, SERINE, $\beta$ -CHLOROALANINE

HEINO SUSI, D. MICHAEL BYLER and WALTER V. GERASIMOWICZ  
*Eastern Regional Research Center\*, Philadelphia, PA 19118 (U.S.A.)*

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## ABSTRACT

Normal coordinate calculations were carried out involving a total of seven isotopically substituted analogs of the amino acids cysteine, serine, and  $\beta$ -chloroalanine. Raman spectra were obtained for polycrystalline  $\beta$ -chloroalanine and the  $\text{ND}_3$  analog. Overlay calculations were employed to obtain 55 force constants which reproduce 206 observed frequencies of seven molecules with an average error of ca.  $9\text{ cm}^{-1}$ . The valence force field used was based on local symmetry coordinates. Band assignments were based on the potential energy distribution. About 60% of the normal modes of the seven isotopomers can be called group vibrations by the PED criterion. Most skeletal stretching and bending vibrations are highly mixed and cannot be assigned to individual bond stretching or angle deformation modes.

## INTRODUCTION

Vibrational Raman spectra of amino acids were first reported and qualitatively interpreted in a classic paper by Edsall in 1936 [1]. A detailed vibrational analysis of the simplest member, glycine, based on the spectra of seven isotopomers was reported by Destrade et al. [2]. Normal coordinate analyses based on several isotopomers have been reported for alanine in terms of a local symmetry valence force field [3] and a Urey-Bradley force field [4]. The vibrational spectra of the remaining amino acids have been studied in much less detail. For D,L-serine the IR spectra of seven isotopomers and the Raman spectra of four isotopomers have been reported along with tentative group frequency assignments [5]. A simple normal coordinate calculation with transferred Urey-Bradley force constants has been performed for normal serine and the  $d_4$  analog [6]. Raman and IR spectra have been published for the normal and the  $d_4$  isotopomer of L- and D,L-cysteine, along with tentative group frequency assignments [7].

For complex molecules, such as amino acids, the experimental information needed to determine a computationally meaningful force field is frequently not available even with extensive isotopic substitution. For structurally

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\*Agricultural Research Service, U.S. Department of Agriculture.

related molecules empirical force fields and vibrational assignments can nevertheless be obtained by overlay calculations which employ a large number of experimental frequencies from many related molecules to derive force constants which are by definition transferable among the studied species [8-10]. In principle, comprehensive sets of transferable, empirical force constants can thus be evaluated for a large number of molecules [10]. We have carried out overlay calculations involving three isotopomers of D,L-serine:  $\text{HOCH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$ ,  $\text{DOCH}_2\text{CH}(\text{ND}_3^+)\text{COO}^-$ , and  $\text{HOCD}_2\text{CD}(\text{NH}_3^+)\text{COO}^-$ ; two isotopomers of L-cysteine:  $\text{HSCH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$  and  $\text{DSCH}_2\text{CH}(\text{ND}_3^+)\text{COO}^-$ ; and two isotopomers of D,L- $\beta$ -chloroalanine:  $\text{ClCH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$  and  $\text{ClCH}_2\text{CH}(\text{ND}_3^+)\text{COO}^-$ . Although the latter is not a naturally occurring amino acid, its inclusion in the studied series facilitates both the computations and the assignments because it serves as a structural intermediate between the previously studied alanine [3] and the more complex serine and cysteine molecules. We hope that this work facilitates the interpretation of the vibrational spectra of complex molecules of biological origin in general.

#### EXPERIMENTAL PROCEDURE AND SOURCE OF DATA

Orthorhombic L-cysteine was obtained from the Aldrich Chemical Company\* ("Aldrich analyzed") and used after recrystallization from water. The spectra were in good agreement with published data [7]. D,L- $\beta$ -Chloroalanine was purchased from the Sigma Chemical Company (Sigma Research Reagent). S- and N-deuterated analogs were prepared by dissolving the samples in a large excess of  $\text{D}_2\text{O}$  (Aldrich, >99% isotopic purity) and evaporating the resulting solution to dryness in a trap attached to a vacuum line. No isotopic impurities were spectroscopically detectable. L-Cysteine crystallizes in an orthorhombic and a monoclinic form, but only the orthorhombic  $d_4$  isotopomer can be obtained from  $\text{D}_2\text{O}$  solution [7]. Raman spectra of both the normal and N-deuterated orthorhombic cysteine isotopomers have been reported [7], but exact frequencies are not available. We therefore used our own frequency data for both cysteine isotopomers as well as for  $\beta$ -chloroalanine and N-deuterated  $\beta$ -chloroalanine (which have not been previously reported). Whenever possible Raman data were chosen for computations over IR frequencies because of fewer uncertainties due to overtones and combinations.

Raman spectra of polycrystalline solids sealed in melting-point capillaries were obtained from 40 to  $3500\text{ cm}^{-1}$  on a Spex 1401 spectrometer with the 514.5 nm line of a Spectra-Physics Model 165-03 argon ion laser used for excitation. The laser power at the sample was ca. 250 mW. The spectral slit width was  $3\text{ cm}^{-1}$ . The Raman spectra of  $\beta$ -chloroalanine and the  $\text{ND}_3$

\*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

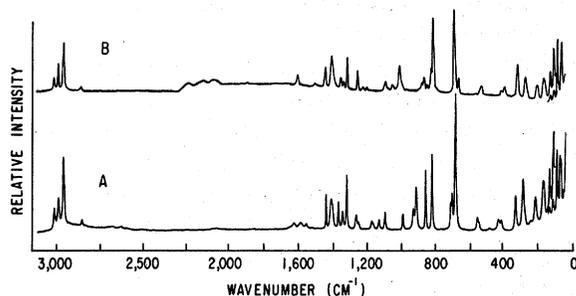


Fig. 1. Raman spectra of polycrystalline  $\beta$ -chloroalanine (A) and  $\beta$ -chloroalanine-ND<sub>3</sub> (B) (corrected for fluorescence).

analog are given in Fig. 1. Auxiliary FTIR data were obtained from 400 to 4000  $\text{cm}^{-1}$  with a Nicolet 7000 series instrument. Vibrational frequencies for the three D,L-serine isotopomers were taken from the work of Madec et al. [5]. Computations were carried out on an IBM 370/168-1 computer.

## COMPUTATIONS

### *Numerical methods and structural considerations*

Numerical calculations were based on the GF-matrix formulation [11] and a least-squares refinement of force constants as previously described [12]. The G-matrices were calculated from structural data obtained by neutron diffraction studies cited in ref. 3. Except for the torsional angles, average values for bond distances and bond angles were used for corresponding fragments of different amino acid molecules, as previously described [3]. The average values agree with the ones for specific molecules within experimental error. Figure 2 gives a schematic structural diagram of the L-cysteine molecule. The structure of serine is in principle analogous, although the dihedral angles have different values. For  $\beta$ -chloroalanine the terminal hydrogen atom is, of course, missing. The following bond-length values (in Ångstroms) were employed: C(2)—O, 1.249; C(2)—C(3), 1.529; C(3)—C(8), 1.524; C—N, 1.485; C(3)—H, 1.098; C(8)—H, 1.084; N—H, 1.037; C(8)—O, 1.413 [3]; O—H, 0.958 [15]; C—S, 1.796; S—H, 1.30 [14]; C—Cl, 1.79 [13]. The bond angles around C(3), C(8), and N were taken to be tetrahedral, as before [3]. The average values for the angles around C(2) are:  $\angle \text{CCO} = 117.23^\circ$  and  $\angle \text{OCO} = 125.55^\circ$  [3].  $\angle \text{COH} = 109.3^\circ$  (serine) [15] and  $\angle \text{CSH} = 97.0^\circ$  (cysteine) [14]. The torsional angles for cysteine and serine were from neutron diffraction data [14, 15]. No structural data are available for  $\beta$ -chloroalanine; therefore cysteine values were applied.

Internal bond-stretching and angle-bending coordinates, as well as torsional coordinates around the C(2)—C(3), C(3)—N, and C(3)—C(8) bonds were defined as for alanine [3]. Torsion around the C—X bond (X = O, S) was

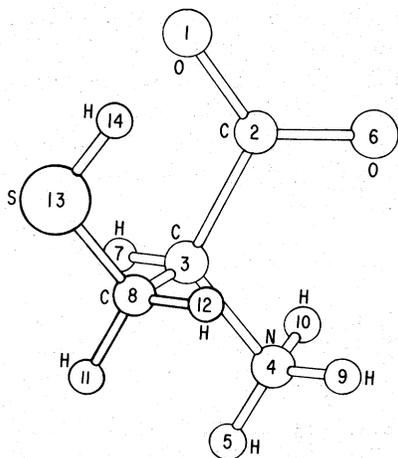


Fig. 2. Schematic molecular structure of cysteine and serine.

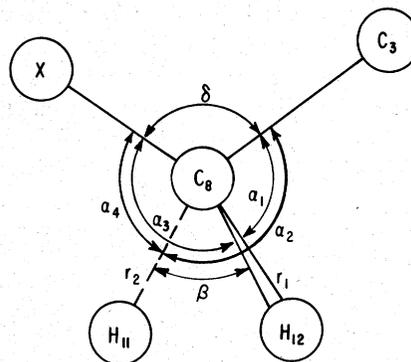


Fig. 3. Internal coordinates for the C—CH<sub>2</sub>—X group.

defined as follows:  $\tau(\text{CXH}) = \tau(14,13,8,3) + \tau(14,13,8,11) + \tau(14,13,8,12)$ . The COO<sup>-</sup> wagging coordinate is defined in terms of the angle between the C(2)—C(3) bond and the plane of the COO<sup>-</sup> grouping. The sign is positive if the C(3) atom in Fig. 2 moves up.

### Symmetry coordinates and force field

Amino acid molecules have no overall symmetry. Linear combinations of internal displacement coordinates corresponding to the local symmetries of the CH<sub>2</sub>, NH<sub>3</sub><sup>+</sup>, and COO<sup>-</sup> groups were nevertheless defined analogously to previous work on glycine [2] and alanine [3]. Such a procedure permits computation of the potential energy distribution (PED) in terms of generally accepted local modes of the CH<sub>2</sub>, NH<sub>3</sub><sup>+</sup>, and COO<sup>-</sup> groups. This procedure also makes it easier to choose significant interaction force constants, and permits comparisons with previously studied amino acids [2, 3].

Local symmetry coordinates for the NH<sub>3</sub><sup>+</sup> and COO<sup>-</sup> groups were identical with the ones for glycine [2] and L-alanine [3]. For the C—CH<sub>2</sub>—X grouping (X = S, O, Cl) the following local symmetry coordinates were defined (see Fig. 3):

$S_1 = 1/\sqrt{20} (4\beta - \alpha_1 - \alpha_2 - \alpha_3 - \alpha_4)$	CH <sub>2</sub> bending
$S_2 = 1/2 (\alpha_1 + \alpha_2 - \alpha_3 - \alpha_4)$	CH <sub>2</sub> wagging
$S_3 = 1/2 (\alpha_1 - \alpha_2 - \alpha_3 + \alpha_4)$	CH <sub>2</sub> twisting
$S_4 = 1/2 (\alpha_1 - \alpha_2 + \alpha_3 - \alpha_4)$	CH <sub>2</sub> rocking
$S_5 = 1/\sqrt{30} (5\delta - \beta - \alpha_1 - \alpha_2 - \alpha_3 - \alpha_4)$	XCC bending
$S_6 = 1/\sqrt{2} (r_1 + r_2)$	CH <sub>2</sub> sym. str.
$S_7 = 1/\sqrt{2} (r_1 - r_2)$	CH <sub>2</sub> antisym. str.

The force constants are defined in terms of the described local symmetry coordinates for the  $\text{COO}^-$ ,  $\text{NH}_3^+$ , and  $\text{CH}_2$  groups, and in terms of bond-stretching and angle-bending coordinates for the central backbone containing the atoms C(2), C(3), C(8), N(4), X(13), H(7), and H(14), (Fig. 2), where X = O, S, Cl. Tables 1 and 2 list the diagonal force constants, the corresponding displacement coordinates, and the chosen interaction force constants in the constrained force field which was adopted.

TABLE 1

Diagonal force constants for cysteine, serine, and  $\beta$ -chloroalanine

Force constant	Local symmetry coordinate <sup>a</sup>	Value <sup>b</sup>	Dispersion <sup>c</sup>
<i>All molecules</i>			
1 <sup>i</sup>	$\nu_a \text{NH}_3^+$	5.369	0.016
2 <sup>i</sup>	$\nu_s \text{NH}_3^+$	5.432	0.036
3	$\delta_a \text{NH}_3^+$	0.618	0.003
4	$\delta_s \text{NH}_3^+$	0.654	0.005
5	$\rho_{  } \text{NH}_3^+$	0.755	0.018
6	$\rho_{\perp} \text{NH}_3^+$	0.724	0.015
7	$\tau \text{NH}_3^+$	0.049	0.001
8	$\nu_a \text{CH}_2$	4.774	0.016
9	$\nu_s \text{CH}_2$	4.962	0.021
10	$\delta \text{CH}_2$	0.545	0.004
11	$\gamma \text{CH}_2$	0.612	0.007
12	$t \text{CH}_2$	0.664	0.012
13	$\rho \text{CH}_2$	0.507	0.022
14	$\tau \text{CC-CX}^h$	0.106	0.011
15	$\nu \text{CH}$	4.741	0.016
16	$\delta \text{CCH, NCH}$	0.648	0.010
17 <sup>d</sup>	$\nu_a \text{COO}^-$	8.500	—
18 <sup>d</sup>	$\nu_s \text{COO}^-$	11.100	—
19	$\delta \text{COO}^-$	1.147	0.046
20	$\rho \text{COO}^-$	1.430	0.050
21	$\gamma \text{COO}^-$	0.607	0.021
22	$\tau \text{COO}^-$	0.070	0.007
23 <sup>d</sup>	$\nu \text{C(3)C(8)}$	4.460	—
24 <sup>d</sup>	$\nu \text{C(2)C(3)}$	4.390	—
25 <sup>d</sup>	$\nu \text{CN}$	3.890	—
26 <sup>e</sup>	$\delta \text{NC(3)C(8)}$	1.345	—
27 <sup>e</sup>	$\delta \text{NC(3)C(2)}$	1.470	—
28 <sup>e</sup>	$\delta \text{CCC}$	1.086	—
<i>Cysteine only</i>			
29	$\nu \text{SH}$	3.768	0.030
30	$\nu \text{CS}$	3.223	0.130
31	$\delta \text{CCS}$	0.581	0.117
32	$\delta \text{CSH}$	0.725	0.029
33	$\tau \text{CSH}$	0.013	0.001

TABLE 1 (continued)

Force constant	Local symmetry coordinate <sup>a</sup>	Value <sup>b</sup>	Dispersion <sup>c</sup>
<i>Serine only</i>			
34 <sup>f</sup>	$\nu$ OH	—	—
35 <sup>d</sup>	$\nu$ C(8)O	4.700	—
36	$\delta$ CCO	1.134	0.098
37	$\delta$ COH	0.766	0.013
38	$\tau$ COH	0.028	0.001
<i><math>\beta</math>-Chloroalanine only</i>			
39	$\nu$ CCl	2.843	0.110
40 <sup>g</sup>	$\delta$ CCCI	0.936	—

<sup>a</sup> $\nu_a$ , antisym. str;  $\nu_s$ , sym. str.;  $\delta_s$ , sym. bend;  $\rho$ , rocking;  $\gamma$ , wagging;  $\tau$ , torsion;  $t$ , twisting. <sup>b</sup>Units: stretching, mdyn  $\text{\AA}^{-1}$ ; bending and torsion, mdyn  $\text{\AA} \text{rad}^{-2}$ . <sup>c</sup>Estimated from the standard error in frequency parameters. <sup>d</sup>Calculated from bond length [3]. <sup>e</sup>Transferred from alanine [3]. <sup>f</sup>Could not be determined. <sup>g</sup>Transferred from alkylchlorides [18]. <sup>h</sup>X = O, S, Cl. <sup>i</sup>Not for  $\beta$ -chloroalanine. See text.

TABLE 2

## Interaction force constants

Force constant	Local symmetry coordinates <sup>a</sup>	Value <sup>d</sup>	Dispersion <sup>e</sup>
41	$\nu$ CN, $\delta_s$ NH <sub>3</sub> <sup>+</sup>	-0.376	0.030
42	$\nu_s$ COO <sup>-</sup> , $\delta$ COO <sup>-</sup>	1.773	0.083
43	$\delta$ COO <sup>-</sup> , $\nu$ C(2)C(3)	-0.320	0.053
44	$\nu_a$ COO <sup>-</sup> , $\rho$ COO <sup>-</sup>	1.289	0.027
45	$\delta$ COO <sup>-</sup> , $\gamma$ COO <sup>-</sup>	0.036	0.020
46	$\nu$ CX, $\nu$ C(3)C(8)	0.227	0.155
47	$\nu$ CX, $\delta$ CXH	0.264	0.035
48	$\nu$ CX, $\delta$ CH <sub>2</sub>	-0.238	0.054
49	$\nu$ CX, $\gamma$ CH <sub>2</sub>	0.370	0.045
50	$\nu$ CX, $\delta$ CCX	0.364	0.047
51	$\nu$ C(3)C(8), $\delta$ CH <sub>2</sub>	-0.197	0.036
52	$\nu$ C(3)C(8), $\gamma$ CH <sub>2</sub>	-0.198	0.034
53 <sup>b</sup>	$\tau$ COH, $t$ CH <sub>2</sub>	0.027	0.004
54 <sup>c</sup>	$\delta$ C(8)C(3)N, $\delta$ CCCI	0.382	0.050
55	$\nu$ skel, $\delta$ CH	0.254	0.013

<sup>a</sup>X = S, O, Cl. <sup>b</sup>Applies to serine isotopomers only. <sup>c</sup>Applies to  $\beta$ -chloroalanine isotopomers only. <sup>d</sup>Units: stretch—stretch, mdyn  $\text{\AA}^{-1}$ ; stretch—bend, mdyn  $\text{\AA} \text{rad}^{-1}$ ; bend—bend, mdyn  $\text{\AA} \text{rad}^{-2}$ . <sup>e</sup>Estimated from the standard error in frequency parameters.

Initial values for diagonal force constants involving functional groups common with alanine were transferred from this molecule [3]. [F(1)—F(7); F(15)—F(28)]. Corresponding values for the  $-\text{CH}_2\text{SH}$  grouping of cysteine were transferred from the work of Scott et al. on ethanethiol [16, 17]. Local symmetry force constants were calculated from the valence force constants given by these authors by applying the symmetry coordinates listed in the previous section. Initial force constants for the OH group of serine were estimated from the assigned frequency values of bending and torsional modes, because no relevant study of valence force constants of related molecules appeared to have been published. (The OH stretching mode could not be distinguished from  $\text{NH}_3^+$  vibrations.) C—Cl stretching and bending constants for  $\beta$ -chloroalanine were taken from alkyl chlorides [18]. Initial interaction force constants for the  $-\text{HC}(\text{NH}_3^+)\text{COO}^-$  group were from alanine [3] [F(41) to F(45) and F(55)], for the  $-\text{CH}_2\text{XH}$  group (X = O, S, Cl) from ethanethiol [15] [F(46) to F(52)]. One specific interaction constant, F(53), was added for serine and another, F(54), for  $\beta$ -Cl-alanine on the basis of preliminary calculations.

The first refinements involved all diagonal force constants except the skeletal stretching constants F(17), F(18), F(23—25) (calculated from bond lengths, as previously described [3]) and skeletal bending constants F(26) to F(28), which were transferred from alanine [3]. Good frequency agreement was obtained. Subsequently, all diagonal force constants were fixed at the calculated values and all interactions were refined. Such a procedure was necessary because even the seven isotopomers did not supply enough data to refine all selected force constants simultaneously. The computational uncertainties given in Tables 1 and 2 refer to these calculations with first all off-diagonal and then all diagonal constants fixed. They do nevertheless provide some indication of the computational significance of the values and the relative empirical importance of the chosen interaction terms.

## RESULTS AND DISCUSSION

Table 1 gives the values of the refined diagonal force constants, and Table 2 lists the values of the interaction force constants. The computational uncertainties are relatively small, particularly for the diagonal terms. Of the 55 force constants, 28 diagonal terms and 13 interactions apply to all studied amino acids and are thus by definition transferable. The OH stretching force constant for serine could not be determined because the corresponding IR and Raman bands could not be distinguished from  $\text{NH}_3^+$  stretching bands. The choice of interaction constants is based on previous work on alanine [3] and ethanethiol [7], except for F(53) (serine only) and F(54) ( $\beta$ -chloroalanine only.) No additional interaction terms could be meaningfully determined on the basis of the available data.

Tables 3 to 9 give the observed and calculated frequencies of the seven

TABLE 3

## Fundamental vibrations on L-cysteine

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
3166	3156	1(99)	} v <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
3166	3155	1(99)	
3064 <sup>d</sup>	3064	2(100)	v <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
2994	2996	8(99)	v <sub>a</sub> CH <sub>2</sub>
2961	2976	9(100)	v <sub>s</sub> CH <sub>2</sub>
2961	2954	15(99)	v CH
2551	2559	29(100)	v SH
1641	1645	3(91)	} δ <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
1641	1642	3(92)	
1575	1588	17(108), 20(13), 44(-27)	v <sub>a</sub> COO <sup>-</sup>
1523	1524	4(100)	δ <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
1424	1439	10(82)	δ CH <sub>2</sub>
1397	1410	24(28), 16(24), 10(18), 18(17)	δ CH
1344	1356	16(65), 25(12), 55(-12)	
1303	1322	16(29), 18(27), 12(22)	γ CH <sub>2</sub>
1269	1278	11(77), 23(19)	
—	1258	12(54), 18(36)	t CH <sub>2</sub>
1198	1180	5(26), 16(23), 11(11)	ρ <sub>1</sub> NH <sub>3</sub> <sup>+</sup>
1140	1144	6(48), 11(15), 5(11)	
1106	1103	25(20), 5(17), 12(12)	γ COO <sup>-</sup>
1004	1008	5(21), 24(19), 6(16)	
931	922	25(15), 21(15), 6(12)	v CS
868	883	13(31), 32(24), 24(12)	
826	827	21(40), 32(27)	δ COO <sup>-</sup>
773	795	32(36), 13(24), 21(12)	
691	687	30(77), 19(19), 32(12)	τ NH <sub>3</sub> <sup>+</sup>
638	635	19(40), 30(20), 42(-17)	
535	535	25(29), 20(25), 26(14)	τ CSH
498	487	7(77)	
441	444	26(31), 20(18), 31(14)	δ NCC
364	358	23(23), 33(20), 19(14)	
298	307	33(54), 27(16)	τ COO <sup>-</sup>
268	271	27(39), 20(23), 33(15)	
210	207	22(56), 31(11)	δ CCS
148	136	22(30), 14(26), 13(16)	
118	111	31(49), 28(23)	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>Raman data obtained in this laboratory. <sup>d</sup>FTIR frequency obtained in this laboratory.

TABLE 4

Fundamental vibrations of cysteine-ND<sub>3</sub><sup>+</sup>, SD

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
2996	2996	8(100)	v <sub>a</sub> CH <sub>2</sub>
2964	2976	9(100)	v <sub>s</sub> CH <sub>2</sub>
2964	2954	15(99)	v CH
2377 <sup>d</sup>	2341	1(98)	} v <sub>a</sub> ND <sub>3</sub> <sup>+</sup>
2377 <sup>d</sup>	2340	1(98)	
2200	2200	2(99)	v <sub>s</sub> ND <sub>3</sub> <sup>+</sup>
1849	1838	29(100)	v SD
1576	1588	17(109), 44(-27), 20(13)	v <sub>a</sub> COO <sup>-</sup>
1426	1437	10(92)	δ CH <sub>2</sub>
1398	1400	18(23), 24(34), 16(30)	δ CH
1358	1356	16(68), 23(12), 55(-11)	
1330	1320	16(32), 18(25), 12(22)	γ CH <sub>2</sub>
1285	1274	11(80), 23(21), 49(-10), 52(-10)	
1246	1257	12(54), 18(34)	t CH <sub>2</sub>
1195	1184	3(76)	} δ <sub>a</sub> ND <sub>3</sub> <sup>+</sup>
1178	1180	3(94)	
1170	1165	4(45), 25(17), 3(17)	δ <sub>s</sub> ND <sub>3</sub> <sup>+</sup>
—	1138	4(48), 16(13), 11(10)	δ <sub>s</sub> ND <sub>3</sub> <sup>+</sup>
1055	1064	18(15), 24(13), 25(12)	ρ CH <sub>2</sub>
1024	1019	6(16), 5(10), 16(7)	
878	873	13(41), 21(13), 14(12)	ρ <sub>  </sub> ND <sub>3</sub> <sup>+</sup>
841	838	5(52), 24(12), 19(24)	ρ <sub>⊥</sub> ND <sub>3</sub> <sup>+</sup>
808	809	21(35), 23(19), 13(11)	
773	789	6(57), 21(13)	v CS
687	695	30(80), 50(-14)	δ COO <sup>-</sup>
628	610	19(44), 20(17), 42(-20)	δ CSD
614	596	32(90), 30(15), 47(-13)	τ ND <sub>3</sub> <sup>+</sup>
508	510	20(29), 25(28), 19(17)	
424	428	26(34), 20(13), 31(17)	δ NC(3)C(2)
354	354	7(67)	
—	337	28(24), 7(23)	τ CSD
276	265	27(53), 20(23)	τ COO <sup>-</sup>
234	220	33(70)	δ CCS
186	195	22(60)	
122	132	14(29), 22(24), 13(17)	
114	109	31(50), 28(22)	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>Raman data obtained in this laboratory. <sup>d</sup>FTIR frequency obtained in this laboratory.

TABLE 5

## Fundamental vibrations of serine

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
— <sup>d</sup>	—	—	v OH
3144 <sup>e</sup>	3156	1(99)	} v <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
3144 <sup>e</sup>	3155	1(99)	
—	3064	2(100)	v <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
2975	2998	8(99)	v <sub>a</sub> CH <sub>2</sub>
—	2978	9(99)	v <sub>s</sub> CH <sub>2</sub>
2945	2954	15(99)	v CH
1648	1645	3(91)	} δ <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
1648	1642	3(92)	
1578	1589	17(108), 20(13), 44(-28)	v <sub>a</sub> COO <sup>-</sup>
1522	1525	4(100)	δ <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
1450	1441	10(81)	δ CH <sub>2</sub>
1435	1414	24(27), 16(24), 10(18)	δ CH
1364	1364	16(63), 23(10), 25(13), 55(-12)	
1352	1328	12(28), 18(22), 16(15)	γ CH <sub>2</sub>
1312	1311	11(51), 16(20), 37(12)	
—	1272	12(31), 18(24), 37(23)	δ COH
1248	1253	37(55), 18(10), 12(19)	
1180	1183	5(26), 18(14), 16(22)	ρ <sub>⊥</sub> NH <sub>3</sub> <sup>+</sup>
1162	1151	6(42), 5(14), 11(14)	
1095	1106	25(22), 5(11)	v CD
1030	1024	35(23), 5(12), 6(22)	
983	994	35(50), 24(10), 5(15)	δ COO <sup>-</sup>
901	903	21(36), 35(15)	
849	851	13(39), 19(13), 38(15)	τ NH <sub>3</sub> <sup>+</sup>
815	827	38(36), 19(19), 21(18)	
728	723	38(37), 13(19), 53(12)	τ NH <sub>3</sub> <sup>+</sup>
618	622	19(48), 27(12), 42(-21)	
566	560	36(36), 20(13), 26(17)	τ NH <sub>3</sub> <sup>+</sup>
525	530	20(31), 25(27), 19(13)	
499	487	7(82)	τ NH <sub>3</sub> <sup>+</sup>
— <sup>f</sup>	(370)	—	
— <sup>f</sup>	(277)	—	τ NH <sub>3</sub> <sup>+</sup>
— <sup>f</sup>	(249)	—	
— <sup>f</sup>	(169)	—	τ NH <sub>3</sub> <sup>+</sup>
— <sup>f</sup>	(162)	—	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>From ref. 5. <sup>d</sup>Not observed in presence of CH and NH modes. <sup>e</sup>Our FTIR values. <sup>f</sup>No data. See text.

TABLE 6

Fundamental vibrations of serine-ND<sub>3</sub>, OD

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
2985	2998	8(99)	v <sub>a</sub> CH <sub>2</sub>
—	2978	9(99)	v <sub>s</sub> CH <sub>2</sub>
2943	2954	15(99)	v CH
— <sup>d</sup>	—	—	v OD
2350 <sup>e</sup>	2341	1(98)	} v <sub>a</sub> ND <sub>3</sub> <sup>+</sup>
2350 <sup>e</sup>	2340	1(98)	
2230 <sup>e</sup>	2200	2(99)	v <sub>s</sub> ND <sub>3</sub> <sup>+</sup>
1592	1589	17(109), 20(13), 44(-28)	v <sub>a</sub> COO <sup>-</sup>
1452	1438	10(92)	δ CH <sub>2</sub>
1424	1403	24(34), 18(22), 16(30)	δ CH
1370	1365	16(67), 23(12), 25(10)	
1340	1324	12(27), 18(25), 16(25)	
1308	1302	11(74), 23(17), 16(12)	γ CH <sub>2</sub>
1277	1263	12(53), 18(32), 16(10)	t CH <sub>2</sub>
1185	1184	3(70)	} δ <sub>a</sub> ND <sub>3</sub> <sup>+</sup>
1180	1180	3(94)	
1165	1167	4(32), 25(14), 3(24), 41(-10)	δ <sub>s</sub> ND <sub>3</sub> <sup>+</sup>
1140	1143	4(57), 11(9), 16(9)	
1095	1079	18(19), 24(12), 16(8)	δ COD
1023	1047	35(20), 6(9), 16(9)	
985	983	37(47), 47(-12), 35(39)	ρ <sub>  </sub> ND <sub>3</sub> <sup>+</sup>
940	933	35(29), 37(29)	
874	847	21(26), 6(10), 13(16), 37(11)	τ ND <sub>3</sub> <sup>+</sup>
848	836	5(58), 19(16)	
808	799	6(21), 23(19), 19(14)	τ ND <sub>3</sub> <sup>+</sup>
782	777	13(27), 21(22), 6(27)	
606	616	19(21), 21(13), 38(20)	τ ND <sub>3</sub> <sup>+</sup>
575	573	19(22), 20(18), 38(21)	
—	518	36(25), 26(19), 38(23)	τ ND <sub>3</sub> <sup>+</sup>
507	505	20(33), 25(22), 19(20)	
365	356	7(48), 19(10), 26(10)	τ ND <sub>3</sub> <sup>+</sup>
— <sup>f</sup>	(346)	—	
— <sup>f</sup>	(259)	—	
— <sup>f</sup>	(229)	—	
— <sup>f</sup>	(167)	—	
— <sup>f</sup>	(158)	—	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>From ref. 5. <sup>d</sup>Not observed because of ND modes. <sup>e</sup>Estimated from drawing in reference [5]. <sup>f</sup>No data. See text.

TABLE 7

Fundamental vibrations of serine-CD<sub>2</sub>, CD

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
— <sup>d</sup>	—	—	v OH
3150 <sup>e</sup>	3156	1(99)	} v <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
3150 <sup>e</sup>	3155	1(99)	
—	3064	2(100)	v <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
2240	2238	8(98)	v <sub>a</sub> CD <sub>2</sub>
2175	2193	15(94)	v CD
—	2177	9(95)	v <sub>s</sub> CD <sub>2</sub>
1652	1645	3(91)	} δ <sub>a</sub> NH <sub>3</sub> <sup>+</sup>
1652	1641	3(92)	
1576	1589	17(109), 20(13), 44(−28)	v <sub>a</sub> COO <sup>−</sup>
1510	1522	4(103)	δ <sub>s</sub> NH <sub>3</sub> <sup>+</sup>
1402	1396	24(38), 18(32), 23(16), 42(13)	δ COH
—	1302	23(31), 18(24), 25(18)	
1265	1269	37(95)	v <sub>s</sub> COO <sup>−</sup>
1212	1195	18(47), 25(16), 5(18), 16(13)	δ CD <sub>2</sub>
1145	1159	5(23), 25(12), 6(18), 35(12)	
1127	1104	6(37), 11(26), 35(28)	δ CD
1058	1052	10(82), 48(−10), 35(13)	
1026	1023	5(28), 6(16), 16(16), 10(14)	τ COH
973	955	16(35), 21(10), 12(14)	
924	921	12(36), 21(12)	ρ CH <sub>2</sub>
915	908	11(31), 49(14), 35(31)	
893	867	16(45), 12(11)	τ NH <sub>3</sub> <sup>+</sup>
836	844	21(33), 16(14), 12(11)	
769	788	19(24), 23(12), 11(23), 38(12)	—
752	747	38(69)	
—	639	13(58)	—
593	604	19(35), 27(10), 20(11), 21(10)	
546	542	20(31), 36(23)	—
512	508	19(22), 25(15), 20(14), 26(13)	
495	482	7(76)	—
— <sup>f</sup>	(346)	—	
— <sup>f</sup>	(272)	—	—
— <sup>f</sup>	(245)	—	
— <sup>f</sup>	(169)	—	—
— <sup>f</sup>	(161)	—	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>From ref. 5. <sup>d</sup>Not observed because of ND modes. <sup>e</sup>Estimated from drawing in reference [5]. <sup>f</sup>No data available. See text.

TABLE 8

Fundamental vibrations of  $\beta$ -chloroalanine

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
— <sup>d</sup>	(3156) <sup>e</sup>	1(99)	} $\nu_a$ NH <sub>3</sub> <sup>+</sup>
— <sup>d</sup>	(3155) <sup>e</sup>	1(99)	
— <sup>d</sup>	(3064) <sup>e</sup>	2(100)	
3015	2996	8(100)	$\nu_s$ NH <sub>3</sub> <sup>+</sup>
2990	2976	9(99)	$\nu_a$ CH <sub>2</sub>
2960	2953	15(99)	$\nu_s$ CH <sub>2</sub>
1626	1645	3(91)	$\nu$ CH
1626	1642	3(92)	} $\delta_a$ NH <sub>3</sub> <sup>+</sup>
1587	1589	17(108), 20(13), 44(-28)	
1540	1525	4(100)	$\nu_a$ COO <sup>-</sup>
1437	1439	10(72)	$\delta_s$ NH <sub>3</sub> <sup>+</sup>
1407	1420	10(28), 18(13), 24(24), 16(21)	$\delta$ CH <sub>2</sub>
1364	1362	16(64), 23(11), 25(13), 55(-12)	$\delta$ CH
1315	1323	18(30), 16(29), 12(21), 14(10)	
1261	1274	11(76), 23(17), 16(10)	$\gamma$ CH <sub>2</sub>
1248	1258	12(55), 18(34)	$t$ CH <sub>2</sub>
1170	1181	5(26), 18(13), 16(25), 11(12)	$\rho_{\perp}$ NH <sub>3</sub> <sup>+</sup>
1126	1141	6(42), 5(19), 11(14)	
1091	1097	25(24), 6(10), 12(12)	
988	1013	5(23), 24(17), 23(11), 6(18)	
907	918	21(27), 25(14), 6(10)	
853	860	13(32), 24(14), 19(18), 21(11)	
815	791	13(30), 21(28)	$\nu$ CCl
681	685	39(63), 19(22), 50(-13), 40(14)	
—	601	19(32), 20(14), 39(30), 42(-15)	
551	532	25(26), 19(12), 20(38)	
480	485	7(82)	$\tau$ NH <sub>3</sub> <sup>+</sup>
427	430	26(58), 16(12), 40(34), 56(-30)	$\delta$ NCC
327	367	19(22), 24(15), 28(14), 26(12)	$\delta$ NCC
283	277	27(53), 20(33)	
212	221	22(42), 14(14), 40(10)	$\tau$ COO <sup>-</sup>
144	144	22(46), 14(13), 40(16)	
—	117	28(31), 13(12), 14(17), 40(30)	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>Raman data obtained in this laboratory. <sup>d</sup>Could not be observed in presence of CH<sub>2</sub> modes by Raman or FTIR.

<sup>e</sup>Does not apply because F(1), F(2) are based on cysteine and serine only.

TABLE 9

Fundamental vibrations of  $\beta$ -chloroalanine-ND<sub>3</sub><sup>+</sup>

Frequency (cm <sup>-1</sup> )		PED <sup>a</sup>	Approximate description <sup>b</sup>
Obs. <sup>c</sup>	Calc.		
3014	2996	8(100)	$\nu_a$ CH <sub>2</sub>
2991	2976	9(99)	$\nu_s$ CH <sub>2</sub>
2961	2953	15(99)	$\nu$ CH
2260 <sup>d</sup>	(2341) <sup>e</sup>	1(98)	} $\nu_a$ ND <sub>3</sub> <sup>+</sup>
2242 <sup>d</sup>	(2340) <sup>e</sup>	1(98)	
2173 <sup>d</sup>	(2200) <sup>e</sup>	2(99)	$\nu_s$ ND <sub>3</sub> <sup>+</sup>
1601	1589	17(109), 20(13), 44(-28)	$\nu_a$ COO <sup>-</sup>
1437	1436	10(91)	$\delta$ CH <sub>2</sub>
1402	1409	18(20), 24(33), 23(11), 16(29)	
1347	1363	16(67), 23(12), 55(-11)	$\delta$ CH
1310	1321	16(30), 18(28), 12(21), 42(-10)	
—	1271	11(78), 23(19)	$\gamma$ CH <sub>2</sub>
1248	1256	12(55), 18(32)	$t$ CH <sub>2</sub>
1186	1183	3(81)	} $\delta_a$ ND <sub>3</sub> <sup>+</sup>
1186	1180	3(94)	
1167	1164	4(45), 25(16), 3(12), 41(-13)	$\delta_s$ ND <sub>3</sub> <sup>+</sup>
—	1140	4(49), 16(13)	$\delta_s$ ND <sub>3</sub> <sup>+</sup>
1086	1065	18(17), 24(14), 25(10)	
1004	1010	6(15), 25(10), 21(11), 5(10)	
859	865	13(30), 19(11), 21(23), 14(10)	
840	834	5(53), 24(10), 19(20)	$\rho_{\parallel}$ ND <sub>3</sub> <sup>+</sup>
810	791	6(57), 23(17)	$\rho_{\perp}$ ND <sub>3</sub> <sup>+</sup>
—	783	21(36), 13(29)	
675	672	39(76), 19(13), 50(-16), 40(17)	$\nu$ CCl
—	583	19(36), 20(16), 42(-17), 39(20)	
527	516	20(38), 25(26), 19(12)	
402	412	26(50), 16(12), 40(36), 54(-29)	$\delta$ NCC
—	362	19(19), 24(12), 28(14), 26(17)	
314	348	7(88)	$\tau$ NH <sub>3</sub>
270	260	27(56), 20(29)	$\delta$ NCC
204	208	22(46), 14(11)	$\tau$ COO <sup>-</sup>
162	142	22(42), 14(14), 40(18)	$\tau$ COO <sup>-</sup>
126	116	28(31), 13(13), 14(18), 40(28)	

<sup>a</sup>The first number designates the force constant, the second number (in parentheses), its % PED. <sup>b</sup>See Tables 1 and 2 for designation of symbols. <sup>c</sup>Raman data obtained in this laboratory. <sup>d</sup>Our FTIR values. <sup>e</sup>Does not apply because F(1), F(2) are based on cysteine and serine only.

investigated isotopomers, the potential energy distribution (PED) [19], and a group frequency assignment, where appropriate. To avoid confusion, generally not more than the four largest terms having values greater than 12% are included in the potential energy distribution. If the PED contribution to one local symmetry force constant is over 40%, an approximate group frequency description is given in the fourth column of the tables. Despite the size and general asymmetry of these molecules, a fair number of fundamental modes may be described as approximating group vibrations by this criterion. The average frequency error is  $\sim 9 \text{ cm}^{-1}$  or 1.3% ( $\sim 0.8\%$  if modes below  $400 \text{ cm}^{-1}$  are not counted; see below).

In addition to the reasons for frequency errors inherent in this type of calculation — constrained force field and harmonic approximation — there are three additional potential sources of error: first, the crystal structure changes from one amino acid to another and involves strong hydrogen bonding [14, 15, 20]. Vibrations involving hydrogen-bonded  $\text{COO}^-$  and  $\text{NH}_3^+$  groups thus show noticeable frequency differences from one molecule to another which are not determined solely by the intramolecular force field. A second possible source of frequency errors is the assumption that  $\text{CH}_2$  group force constants are transferrable for molecules having this group attached to O, S, and Cl atoms as in serine, cysteine, and  $\beta$ -chloroalanine. (The characteristic  $\text{CH}_2$  group frequencies show marked changes from one molecule to another, as evidenced in Tables 3–9.)

A third major source of possible errors is the coupling of low frequency internal modes with intermolecular vibrations, in particular for serine where the vibrations of the  $\text{OH} \cdots \text{O}$  hydrogen bonds occur in the same frequency region as low frequency intramolecular modes. Only very incomplete information about low frequency modes is included in the literature data used for the serine isotopomers [5]. Our own preliminary Raman data for normal serine showed seven distinct lines between 150 and  $400 \text{ cm}^{-1}$  (175, 191, 223, 252, 302, 327,  $395 \text{ cm}^{-1}$ ) where five intramolecular vibrations are expected (Table 5). The seven lines must correspond to the five internal modes and two  $\text{H} \cdots \text{O}$  hydrogen bond vibrations. No assignment appears possible because of extensive coupling. Low-frequency modes of serine isotopomers were therefore not included in the refinements. The calculated values are given in parentheses in Tables 3–9, without assignment. For cysteine and  $\beta$ -chloroalanine isotopomers, where intermolecular hydrogen bonding is much weaker, reasonable agreement between calculated and observed frequencies was obtained in the 150– $400 \text{ cm}^{-1}$  region. (The percent error is, of course, relatively high at low frequencies even if the wavenumber error is around the average  $9 \text{ cm}^{-1}$ .) The neglect of bend–bend interactions also has a maximum effect in this wavelength range where skeletal bending modes occur. Figure 4 provides a graphical representation of the distribution of the calculated frequency errors. The overwhelming majority (ca. 85%) of the individual errors are below  $15 \text{ cm}^{-1}$ . The few calculated modes with errors larger than  $25 \text{ cm}^{-1}$  involve ill-defined skeletal vibrations.

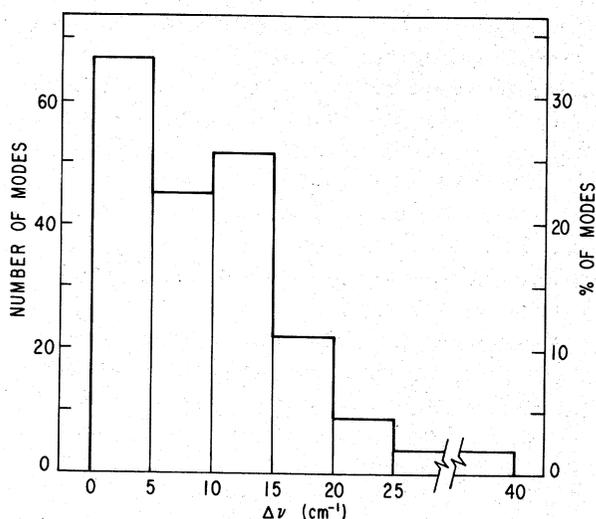


Fig. 4. Distribution of calculated frequency errors.

The assignment of some high-frequency XH stretching modes ( $X = C, N, O$ ) is tentative; others cannot be assigned at all because they overlap with other modes or are not experimentally observed. The latter is the case for the OH and OD stretching modes of serine isotopomers, which cannot be distinguished from CH and NH (or CD and ND) stretching modes [5].  $NH_3^+$  and  $ND_3^+$  stretching modes are generally very weak in the Raman effect; some auxiliary FTIR frequencies were therefore used in the calculations. For normal  $\beta$ -chloroalanine these vibrations could be observed in neither the IR spectra nor the Raman effect. For  $\beta$ -chloroalanine- $ND_3^+$ , infrared  $ND_3^+$  stretching bands were observed, but occurred at substantially lower frequencies than in the other molecules, presumably because of different hydrogen bonding caused by a different crystal structure. The assignments of CH stretching modes were based on  $\beta$ -chloroalanine, alanine [3], and chloroethane [13] spectra. The symmetric  $CH_2$  stretching mode appears to be very weak, or is not observed at all, in cysteine and serine isotopomers, as previously reported [5, 7].

All modes above ca.  $1430\text{ cm}^{-1}$  can be classified as group frequencies by the PED criterion (generally more than 80% pure). Of the remaining modes, deformation vibrations involving XH and XD bonds ( $X = C, O, S, N$ ) are in general identifiable, with the exception of some  $NH_3^+$  and  $ND_3^+$  rocking vibrations, which tend to couple strongly with skeletal modes. C-Cl and C-S stretching modes can be clearly identified along with the antisymmetric  $COO^-$  stretching vibration. The  $NH_3^+$  ( $ND_3^+$ ) torsion is also a clearly distinguished group vibration.

The remaining modes are highly mixed. This holds in particular for skeletal modes involving CC and CN bonds, as well as for the symmetric  $COO^-$

stretching vibration. Attempts to assign all vibrations of complex molecules on a group-frequency basis must be regarded as unjustified oversimplifications. Of the total of 231 investigated fundamental modes, 145 (or ca. 60%) could be called essentially localized bond-stretching or deformation modes, by our PED criteria.

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