[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

Raman Spectra of Amino Acids and Related Compounds. XII. Various Amino Acids Derived from Proteins and Creatine[1,2]

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Raman spectra are reported for serine, threonine, proline, hydroxyproline, valine, leucine, isoleucine, lysine, arginine, creatine and methionine. All were studied in the form of the respective cations, and most in the dipolar ion form as well, but isoelectric serine, leucine, isoleucine and creatine were too insoluble to study in solution as dipolar ions. Proline and hydroxyproline were studied as cation, dipolar ion and anion, and some marked changes, especially in the C–H stretching frequencies, were noted when the dipolar ion was converted to the anion. The spectrum of cationic creatine shows some resemblance to that of the methylguanidinium ion, but that of the arginine cation shows very little. Certain other correlations between spectra and structure are pointed out.

In preceding papers of this series we have studied the Raman spectra of cysteine,[4] which has an ionizing group (sulfhydryl) of particular significance, and of glycine and α- and β-alanine,[5] which are sufficiently simple molecules to permit some theoretical analysis. In this paper we shall consider the spectra of a variety of other amino acids which are important because of the part they play in protein structure, but which are too complicated to permit much theoretical analysis at present. They may be divided into five categories: (1) the hydroxyamino acids, serine and threonine; (2) the imino acids, proline and hydroxyproline; (3) amino acids with non-polar side chains: valine, leucine and isoleucine; (4) basic amino acids: lysine and arginine; (5) the sulfur-containing amino acid, methionine. The Raman spectrum of creatine (methylguanidine acetic acid) will be considered with this group of amino acids, even though it is not an amino acid, because of its close relation to arginine. The spectrum of histidine has been considered in a previous paper.[6]

Experimental

The techniques of obtaining the Raman spectra have been described.[6]

The amino acid solutions studied were clarified and freed of fluorescent impurities by treatment with charcoal at slightly acid *p*H. Alkaline solutions were prepared from the acid ones using Millipore filters[7] for clarification. Proline and hydroxyproline slowly decompose on standing, especially when exposed to intense light, to yield yellow fluorescent products. To remove these it was necessary to boil with charcoal, and a solution allowed to stand several days had to be treated with charcoal again before further study. Fluorescent impurities present in lysine, arginine and creatine were removed by treatment with Amberlite XE-67 anion-exchange resin: the amino acid was dissolved in water, stirred for 45 minutes with resin which had been thoroughly washed with HCl and then with water, and then crystallized by addition of ethanol and ether (this step was omitted for creatine). It seems likely that picric acid is the impurity removed from lysine, and flavianic acid the impurity removed from arginine, as these are commonly employed in the respective isolation of these compounds. The nature of the impurity removed from creatine is unknown. Concentrations (reported as weight/volume, based on the isoelectric compound) of the solutions used for measurement of spectra were: serine hydrochloride, 29% (isoelectric serine is too insoluble to study); threonine hydrochloride, 25%; isoelectric threonine, 15%; proline hydrochloride, 36%; isoelectric proline, 45%; proline sodium salt, 37%; hydroxyproline hydrochloride, 23%; isoelectric hydroxyproline, 21%; and hydroxyproline sodium salt about 15% (using material recovered from the other hydroxyproline solutions, and with KI added as a fluorescence quencher).

The concentrations of the solutions of the larger amino acids, used for measurement of spectra were: valine, 7%; valine hydrochloride, 22%; leucine hydrochloride, 16%; isoleucine hydrochloride, 19%; lysine monohydrochloride, 22%; lysine dihydrochloride, 17%; arginine monohydrochloride, 30%; arginine dihydrochloride, 26%; creatine, 14%; methionine hydrochloride, 29%. The isoelectric forms of leucine, isoleucine and methionine were too insoluble to study. All of the above were clarified by filtration through Whatman No. 4 paper with charcoal. Methionine sodium salt was prepared by making a 30% solution of the hydrochloride with 2.5% KI, filtering with charcoal, as above, adding sufficient NaOH to titrate it to the sodium salt, and then filtering through Whatman No. 4 paper to remove any dust added with the NaOH.

The lysine and arginine were obtained from Merck & Co. and from the Mann Laboratories, respectively. All the other compounds were obtained from the California Foundation for Biochemical Research, Los Angeles. The amino acids were checked for purity chromatographically.[8] All except methionine were free of any ninhydrin-reactive impurity. The leucine preparation was free of isoleucine and *vice versa*. A trace of an impurity which moved like serine was found in the methionine. However, even the strongest serine line is missing from the methionine spectra, and it is believed that this small trace of impurity has not contributed any false lines to the methionine spectrum.

Results and Discussion

Serine.—The Raman spectrum of serine hydrochloride is recorded in Table I. The line at 1735 can be assigned to a CO stretching motion of the

TABLE I[a]

RAMAN SPECTRUM OF SERINE HYDROCHLORIDE, $[HOCH_2{\cdot}CH(NH_3^+){\cdot}COOH]Cl^-$

680(vw), 741(vw), 780(2), 811(vw), 831(3b), 858(w), 907(1b), 974(2b), 1035(1b), 1093(w), 1134(vw), 1156(vw), 1250(1b), 1305(w), 1359(vw), 1401(vw), 1468(3), 1735(1b), 2870(vw), 2896(1b), 2910(2b), 2969(5b)

[a] Intensities were estimated visually and reported as 1,2,3... for increasing intensities, except that the faintest are reported as weak (w) or very weak (vw). Broad lines are designated b, very broad as vb. No correction has been made, in this or the subsequent tables, for concentration of the amino acid solution from which the spectrum was obtained.

(1) This paper is taken from the Ph.D. thesis of David Garfinkel, Graduate School of Arts and Sciences, Harvard University, 1955.

(2) Supported in part by a grant (NSF-G621) from the National Science Foundation to J. T. Edsall.

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(4) D. Garfinkel and J. T. Edsall, THIS JOURNAL, **80**, 3823 (1958).

(5) M. Takeda, *et al.*, *ibid.*, **80**, 3813 (1958).

(6) D. Garfinkel and J. T. Edsall, *ibid.*, **80**, 3807 (1958).

(7) Millipore Filter Corp., Watertown, Mass.

(8) The assistance of Dr. R. H. McMenamy is gratefully acknowledged.

carboxyl group, and that at 1035 is likely to be due to a C–O–H deformation frequency of the alcoholic hydroxyl (based on the finding[9] of such a deformation frequency in alcohols at 1020 cm.$^{-1}$). The line at 1468 cm.$^{-1}$ can be assigned to a CH deformation motion. The 2896 frequency may be due to stretching of the tertiary C–H bond on the α-carbon atom, those at 2910 and 2969 to the methylene stretching motions of the $-CH_2OH$ group.

Threonine.—The Raman spectra of threonine are listed in Table II. It is noteworthy that the line at 1047 cm.$^{-1}$, assigned to an OH deformation motion, appears in the infrared spectra of threonine,[10] in aqueous solution (as well as in the solid) but not in the infrared spectrum of threonine in D_2O. This is to be expected if the given assignment is correct, since the hydrogen on the alcoholic hydroxyl exchanges readily with deuterium. The spectra of both serine and threonine show only one CH deformation line, in contrast to the larger amino acids which have two.

TABLE II

RAMAN SPECTRUM OF THREONINE $HOCH(CH_3)CH(NH_3^+)$-COO^-

Isoelectric	Hydrochloride	Assignments
492(vw)	497(vwb)	
	559(vwb)	
	623(vwb)	
	748(2b)	
776(1b)		
805(vwb)		
	835(1)	
851(wb)		
868(wb)	864(vwb)	
894(vwb)	886(vwb)	
934(1b)	932(2b)	
998(vwb)	1001(1b)	
1047(wb)	1047(2)	OH deformation
	1057(wb)	
1106(wb)	1104(1b)	
	1121(w)	
1252(vw)		NH_3^+ deformation??
1349(2b)	1348(vwb)	COO^- motion, in part
1405(3b)		COO^- symm. stretching
1457(2)	1460(2b)	CH deformation
	1736(2)	CO stretching
1764(?)[a]		
2890(1b)	2894(2b)	
2916(vwb)	2919(1)	
2945(5b)	2945(5)	CH stretching
2993(4b)	2991(4)	

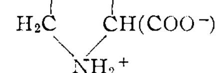
[a] This region is obscured by a false line. There may be a real Raman line here.

Proline and Hydroxyproline.—The Raman spectra of these imino acids are shown in Tables III and IV, respectively. Both show distinct alterations of the spectra when the dipolar ion is converted to the anion by the removal of a proton from the >NH_2^+ group. Even the CH stretching and deformation frequencies seem to be markedly changed, although the changes are not as striking as in glycine or β-alanine.[5]

(9) J. R. Quinan and S. E. Wiberley, *J. Chem. Phys.*, **21**, 1896 (1953).
(10) R. E. Gore, R. B. Barnes and E. Petersen, *Anal. Chem.*, **21**, 383 (1949).

TABLE III

RAMAN SPECTRUM OF PROLINE,



Hydrochloride	Isoelectric	Na salt	Assignments
451(vwb)	455(wb)	446(vw b)	Skeletal deformation??
562(vw)	550(vwb)		
	655(vwb)		
	681(vwb)	683(?)	
755(vwb)		737(vwb)	
	784(vwb)		
822(wb)		823(vwb)	
836(wb)	836(vwb)		
856(wb)	856(1b)	867(wb)	Skeletal stretching??
902(3b)	905(3)	904(wb)	Ring vibration?
		914(3)	
946(vwb)	948(vwb)		
988(wb)	984(vwb)	971(vwb)	
	1024(vw)	1024(vwb)	
1038(2b)	1038(2b)		Ring vibration?
1055(vwb)	1050(vw)		
1092(vwb)	1090(wb)	1094(vwb)	
	1116(vw)		
1184(1b)	1179(1b)	1178(1b)	
	1224(vw)		
1244(wb)	1237(wb)	1237(wb)	
	1276(vwb)	1265(vwb)	
1313(wb)		1302(wb)	
1338(vwb)	1330(1b)	1337(wb)	
1360(vw)	1346(wb)	1362(vwb)	COO^- motion?
		1385(3)	Ring vibration?
1394(vwb)	1398(wb)		COO^- symm. stretching
	1412(vw)		
		1428(vwb)	
1455(4)	1453(4)	1447(3)	CH_2 deformation
		1472(wb)	
1727(wb)			CO stretching
		2837(vwb)	
2891(3)	2888(2b)	2877(4b)	
2916(vw)	2915(vwb)		
2944(4)	2947(3b)	2928(3b)	
2967(1b)		2978(5b)	CH_2 asymm. stretching??
2998(6b)	2994(5b)		
3027(1b)	3026(vwb)		

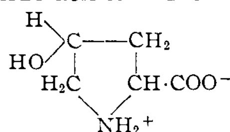
Also noteworthy is the behavior of vibrations of the carboxyl group. The frequency of the carbonyl stretching motion, in the un-ionized carboxyl group of the cations, is about 10 cm.$^{-1}$ lower here than with the typical small amino acids. This may indicate that an imino group affects the vibrations of the carboxyl group less than an amino group. The COO^- symmetrical stretching frequency, in the dipolar ion and anion, does not behave normally. In hydroxyproline the frequency is low; in proline this line is faint; in the spectra of both it is apparently displaced downward, to 1385 in proline and to 1391 in hydroxyproline, when the imino group loses its positive charge, and a weak line appears at 1428 cm.$^{-1}$ also.

Valine, Leucine and Isoleucine.—These amino acids are rather insoluble in the isoelectric form; only the spectrum of valine could be determined in the form of the dipolar ion. Leucine and isoleucine were studied in the cationic form. The spectra are given in Table V.

The spectra of these three amino acids are very similar, as is to be expected. The largest differences are those between valine and isoleucine. The CH stretching lines are strong and numerous, closely resembling those of hydrocarbons, as would be expected from the structure of these amino acids.

TABLE IV[a]

RAMAN SPECTRUM OF HYDROXYPROLINE



Hydrochloride	Isoelectric	Na salt	Assignments
	408(vwb)		
	424(vw)		
	475(vwb)		
	544(vwb)		
		566(vwb)	
	703(vw)	694(vw)	
		719(vw)	
734(vwb)			Sensitive frequency??
	749(vwb)		
765(vwb)	771(vwb)	785(vw)	
	831(vw)		
843(3b)	843(1)		Ring vibration, in part?
	849(4)		
	856(2b)	855(1b)	
873(3b)	874(2b)		Ring vibration
915(vw)			
		930(vw)	
952(vwb)	954(1b)		
		967(w)	
		987(vw)	
1017(vwb)	1016(wb)		OH deformation??
		1051 (vwb)	Ring vibration or NH_2^+ deformation??
1061(1b)	1061(2b)		
		1085(vw)	
		1109(vw)	
1126(vw)			
1184(wb)	1178(wb)	1180(wb)	
1214(2b)	1214(1b)	1219(wb)	
		1253(vw)	
1284(wb)	1272(wb)	1278(vw)	
1320(1b)	1320(3)		
		1330(1b)	Ring vibration?
1358(vwb)	1357(vwb)		
		1391(2)	COO^- symm. stretching
	1401(3b)		
1410(vw)			
		1429(wb)	
1448(1b)	1450(2b)		CH_2 deformation
1652(2 band)	1650(3 band)	1657(3 band)	Water band
1730(2b)			CO stretching
		2887(2b)	
2904(vwb)	2914(vw)		
		2922(w)	
	2951(3b)	2951(3b)	CH_2 symm. stretching??
2962(3b)			
2986(5)	2984(5)	2993(4b)	CH_2 asymm. stretching??
3005(2b)			
3026(3b)	3024(wb)		

[a] The hydroxyl and carboxyl groups of hydroxyproline are *trans* with respect to the plane of the pyrrolidine ring (see A. Neuberger, *Advances in Protein Chem.*, 4, 295 (1948)); J. Donohue and K. N. Trueblood, *Acta Cryst.*, **5**, 419 (1952)).

Lysine, Arginine and Creatine.—Lysine and arginine were studied both as the doubly charged cations, with the carboxyl group un-ionized, and as the singly charged cations, containing two positively charged groups and the anionic $-COO^-$ group. The more basic forms were not studied, because of the technical difficulties of working in alkaline solution.

The spectra of lysine, given in Table VI, show strong C–H stretching frequencies. Indeed these compare in intensity with those of leucine and isoleucine. Otherwise, however, although the observed lines are numerous, few are intense, and assignments are difficult, except for the characteristic

TABLE V[a]

RAMAN SPECTRA OF VALINE, LEUCINE AND ISOLEUCINE

Valine, $(H_3C)_2 \cdot CH \cdot CH(NH_3^+) \cdot COO^-$; leucine, $(H_3C)_2 \cdot CH \cdot CH_2 \cdot CH(NH_3^+)COO^-$; isoleucine, $H_3C \cdot CH_2 \cdot CH(CH_3) \cdot CH(NH_3^+) \cdot COO^-$

Valine cation	Isoelectric valine	Leucine cation	Isoleucine cation	Assignments
		310(vw)		
		330(vw)		
358(vw)	345(vw)		357(vw)	
409(vw)				
438(vw)		430(vw)	438(vw)	
		451(w)		
483(vw)	481(vw)		484(vw)	
539(w)	535(vw)	537(vw)	540(vw)	Skeletal deformation??
640(vw)	636(vw)		642(vw)	
	658(vw)	654(vw)	663(vw)	
729(2)		730(1)	739(2vb)	
742(1)				"Sensitive frequency"?
	758(1b)	755(vw)	760(vw)	
	774(vw)	781(w)		
815(3)		819(2)	812(3)	
	825(w)	827(2)		
842(1)	853(vw)	843(2)		
			867(1b)	
878(1)		886(w)		
	889(w)			
		919(vw)	919(2b)	
941(4b)	943(1b)	943(vw)		
966(1)	965(vw)	958(3)	968(vw)	
			989(1)	
1005(vw)			1000(vw)	
			1016(vw)	
1028(vw)		1041(vw)	1037(w)	
1070(w)	1065(w)		1072(w)	
1127(3b)	1122(w)	1129(4)	1131(2b)	
1165(vw)		1168(2)	1171(w)	
1195(w)	1198(vw)		1200(vw)	
	1237(vw)			
			1253(vw)	
1282(w)	1270(vw)	1283(vw)	1275(vw)	
		1310(vw)	1308(2)	
1325(2b)	1326(3b)	1326(vw)		CH_2 wagging or twisting
1350(2b)	1354(3)	1342(3)	1355(2b)	
1371(vw)				
1398(vw)			1396(vw)	
1414(vw)	1409(3b)			νCOO^- symm. (in part)
1445(5)	1447(2)	1447(5)	1446(5)	δCH_3 and CH_2
1468(5)	1466(3)	1462(4)	1463(5)	
1729(3vb)		1733(2vb)	1733(3vb)	CO stretching
2742(1)		2731(w)	2755(vw)	
2788(w)		2774(w)		
2877(4)	2878(2)	2876(6)	2885(4)	νCH_3 symm.?
2903(5)	2906(3)	2900(3)	2904(2)	νCH_2 at least in part
2921(2b)	2922(vw)	2915(2)	2920(2)	
2941(6b)	2947(4)	2933(4b)	2946(6)	
2979(7)	2980(5b)	2971(7b)	2983(5b)	νCH_3 asymm.

[a] Intensities were estimated visually and are reported as 1,2,3... for increasing intensities, except that the faintest are reported as weak (w) or very weak (vw). Broad lines are designated b, very broad vb.

TABLE VI

RAMAN SPECTRUM OF LYSINE, $^+H_3N(CH_2)_4CH(NH_3^+) \cdot COO^-$

Lysine dihydrochloride: 546(vw), 651(vw), 728(vw), 800(vw), 831(w), 897(vw), 932(vw), 956(w), 1004(1b), 1029(w), 1065(2vb), 1142(w), 1185(vw), 1231(vw), 1323(3), 1339(1b), 1440(4), 1459(1), 1730(2b), 2774(w), 2876(4), 2911(1), 2934(6), 2948(3), 2976(4), 3003(1b)

Lysine monohydrochloride: 549(vw), 810(vw), 840(w), 904(w), 931(vw), 954(w), 1006(w), 1054(1b), 1070(1b), 1152(vw), 1287(vw), 1323(3b), 1348(3b), 1406(2), 1439(4), 1462(vw), 2873(3b), 2908(1), 2929(6), 2949(1), 2976(3b), 3000(w)

C–H stretching and bending frequencies, and the lines associated with the ionized or un-ionized carboxyl group. The carboxyl group is un-ionized in the dihydrochloride, ionized in the monohydrochloride; the two amino groups are positively charged in both cases.

The spectrum of arginine is shown in Table VII,

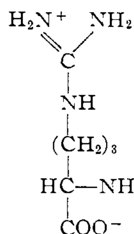
TABLE VII

RAMAN SPECTRUM OF ARGININE

Monohydrochloride	Dihydrochloride	Infrared	Assignments
	338(vw)		
420(vw)			
529(wb)	525(1b)		Guanidine group or skeletal deformation??
602(vwb)	601(vwb)		
	643(vwb)		
		680(m)	
		699(m)	
		722(m)	CH_2 rocking?
	736(vw)		
	758(vwb)		
		786(m)	
	817(vwb)	816(m)	
856(vwb)	851(vw)	845(m)	
880–		884(w)	
980			
(w band)		918(w)	
	905(vw)		
	928(w)		
980	978(w)	974(m)	
	1004(vw)		
1014(w)			
	1031(vwb)	1034(w)	
1048(1)	1052(2b)	1062(w)	Skeletal stretching??
1091(2b)	1090(2b)		Guanidine group motion??
	1103(vw)		
		1136(m)	
1171(1b)	1170(2b)		Guanidine group motion??
		1185(m)	
	1215(wb)		
1250(vw)	1258(wb)	1250(m)	NH_3^+ deformation?
1280(w)			
1319(3b)	1321(2b)	1328(m)	(CH_2 bending)
1354(3b)			COO^- motion
	1368(2b)		
1410(3)		1420(m)	COO^- symm. stretching
1441(4)	1444(4b)		CH_2 deformation
1465(1b)	1464(2b)		Guanidine group motion??
		1511(m)	(NH_3^+ bending)
		1550(s)	COO^- asymm. stretching?
		1613(s)	(NH_3^+ bending)
1644(3 band)	1648(3 band)		Water band
		1684(m)	NH_3^+ deformation?
	1736(2b)	1739(m)	CO stretching
		2597(m)	NH_3^+ motion
		2755(m)	
		2857(m)	(CH stretching)
2892(2b)	2889(2b)[a]		CH_2 symm. stretching??
	2902(3)[a]		
2934(5)[a]			
2949(4b)[a]	2944(5b)		CH_2 asymm. stretching??
2974(2b)	2974(2b)		
		3030(m)	(CH stretching)
		3155(s)	NH_3^+ stretching

[a] These are doublets which appear barely resolvable.

The cation of the monohydrochloride of arginine has the formula



The cation of the dihydrochloride differs in having an unionized carboxyl group. In both forms the positive charge on the guanidinium group is distributed over the three nitrogens of this group, due to resonance.

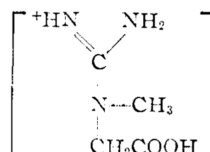
In the infrared spectrum, s denotes strong, m medium and w weak.

TABLE VIII

RAMAN SPECTRUM OF CREATINE HYDROCHLORIDE[a]

433(vw), 534(vw), 597(vw), 672(vw), 803(vw), 824(5), 890(1b), 973(1b), 1049(3b), 1110(vw), 1183(vw), 1329(w), 1423(1), 1440(vw), 1460(w), 1726(1b), 2836(1b), 2905(w), 2951(4b), 2989(w)

[a] The formula of creatine hydrochloride may be written



The resonance between C–N bonds in the guanidinium group is similar to that in arginine.

TABLE IX

RAMAN SPECTRUM OF METHIONINE, $H_3C{\cdot}S{\cdot}(CH_2)_2{\cdot}CH(NH_3^+){\cdot}COO^-$

Hydrochloride	Sodium salt	Infrared	Assignments
315(vwb)			
462(vw)	460(vwb)		Skeletal deformation??
	500(vwb)		
539(wb)	547(1b)		Skeletal deformation??
606(vw)	606(vwb)		
	639(vwb)		
652(4)	651(2)		
674(vw)	675(w)		
696(4)	699(4)	702(w)	C–S stretching
720(4)	722(4)	715(m)	C–S stretching
750(vw)	757(1b)	760(m)	
796(wb)		785(m)	
	882(1b)	875(m)	
908(vwb)			
	919(vw)	918(m)	
947(wb)		950(m)	
985(vwb)	980(vwb)	990(m)	
1031(vwb)		1035(w)	
	1049(1b)		
1072(1)		1080(s)	
	1106(wb)		NH_2 motion?
	1121(vw)		
	1136(vw)		
1151(vwb)	1154(vwb)	1150(m)	
	1203(wb)	1210(s)	
1240(vwb)			NH_3^+ deformation??
		1260(s)	
	1276(1b)		
1290(vwb)			
	1301(1)		
1329(wb)	1331(2)	1330(s)	CH_2–S deformation
1360(wb)	1357(3b)		COO^- motion, in part
		1370(m)	
	1402(wb)	1400(s)	COO^- symm. stretching
1423(5)	1423(5)		CH_2 and CH_3 deformation
1438(4)	1439(4)	1440–50(s) (double)	
		1510(m)	(NH_3^+ deformation)
		1575(s)	(COO^- asymm. stretching)
		1605(m)	
		1625(m)	
1641(3 band)	1655(3 band)		Water band
		1680(s)	(NH_3^+ deformation)?
1737(3b)			CO stretching
		2300(w)	
		2600(m)	NH_3^+–COO^- motions??
		2700(m)	
2843(1b)	2842(2b)	2850(s)	
2870(1b)	2873(2b)		
2928(7)	2927(8)		CH_3 stretching
	2947(1)	2950(s)	
2963(3b)			
2998(4b)	3000(4b)		
		3150(m)	(NH_3^+ stretching)
	3308(4)		Symm. NH_2 stretching
	3365(1)		Asymm. NH_2 stretching

the infrared spectrum[11] being given for comparison. In this case a well-studied functional group (guanidinium) is present; comparison with the spectrum of a fairly close analog of arginine, the methylguanidinium ion,[12,13] shows little similarity. Very few guanidinium group motions can be suggested even tentatively. This indicates that the vibrations of the guanidinium group are strongly coupled with those of the rest of the molecule.

The Raman spectrum of creatine (methylguanidine acetic acid), in the form of the hydrochloride, is given in Table VIII. This spectrum bears more resemblance to that of the methylguanidinium ion than does the spectrum of arginine. The strong line at 824 may correspond to that of the methylguanidinium ion at 915. Other lines that may be associated with this group are 534, 597, 1049, 1183, 1440 and 1460, although no definite assignments can be made.

Methionine.—The spectra of methionine, given in Table IX, with the infrared spectrum for comparison,[14] show the effect of the presence of a sulfur atom. Of the three possible C–S stretching frequencies (around 700 cm.$^{-1}$) one changes intensity appreciably on going from the hydrochloride to the sodium salt form, and this is left unassigned, as it appears extremely improbable that the C–S stretching motion would be affected by the state of ionization of the amino or carboxyl group

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The Properties of Bovine Serum Albumin in Concentrated Acetic Acid[1]

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Some of the properties of bovine serum albumin in glacial acetic acid have been determined. Most of the measurements have been done in the presence of about 0.5 weight % water since the solubility of the protein is very low under anhydrous conditions. In acetic acid, bovine serum albumin has an intrinsic viscosity of 0.134 dl. g.$^{-1}$, a sedimentation constant of 2.3 S and a specific optical rotation of $-31 \pm 1.5°$ compared to the values of 0.036 dl. g.$^{-1}$, 4.4 S and $-62°$ for the same preparation in aqueous acetate buffer. Bovine serum albumin may be recovered apparently unchanged from acetic acid solutions by lyophilization.

The fact that certain proteins are soluble in nonaqueous solvents has been only recently appreciated.[4–8] The observation that native and heat denatured bovine serum albumin (BSA) have different solubilities, not only in aqueous solutions, but also in glacial acetic acid (GAA) has prompted a more thorough investigation of BSA in the latter solvent.

Experimental

Materials.—BSA from Pentex, Inc., was deionized on an ion-exchange column, filtered through a Corning ultra-fine sintered glass filter and lyophilized. The dry protein was stored at $-5°$. This preparation gave a single symmetrical peak in the ultracentrifuge and had an intrinsic viscosity, $[\eta]$, of 0.036 deciliters g.$^{-1}$ in aqueous acetate buffer (0.05 M sodium acetate, 0.05 M acetic acid and 1.0 M sodium chloride). The specific optical rotation was $-62°$ (λ 589 mμ) and the extinction coefficient at 278 mμ, $E_{1\,\mathrm{cm}}^{1\%}$ 6.63.

Reagent grade glacial acetic acid (GAA) was distilled in a dry atmosphere before use.

Methods. Preparation of Solutions.—BSA dried over phosphorus pentoxide was found to be nearly insoluble in GAA but still freely soluble in water. The presence of small amounts of water in GAA or in the protein would raise the solubility greatly and accordingly the experiments were carried out on GAA solutions to which just enough water was added so that the protein would dissolve easily (about 0.5 weight % of water).

Viscosity.—Measurements were made in an Ostwald viscometer at 24°. Slight modifications to the viscometer prevented contact between the solutions and the water of the atmosphere.

Sedimentation.—Sedimentation coefficients were calculated from schlieren patterns in the Spinco Model E Ultracentrifuge and were corrected for solvent viscosity and density to water at 20° unless otherwise indicated.

Optical Rotation.—A visual polarimeter (Rudolph #80) was used at 589 mμ with 20 cm cells.

Tryptic Digestion.—The reaction mixtures contained 1% BSA, 0.01% trypsin, 0.1 M tris-(hydroxymethyl)-aminomethane and 0.01 M calcium chloride, pH 8.9 at 24°. At intervals, samples were withdrawn and trichloroacetic acid (TCA) was added to bring the TCA concentration to 10%. The absorption of the TCA filtrate was measured at 278 mμ.

Recovery of BSA from GAA Solution.—It was found that BSA could be effectively recovered from GAA solution by lyophilization. The dry protein thus obtained (designated BSA-AA) dissolved readily in water to give a solution (1% protein) having a pH of 3.8. After several successive lyophilizations from water or after dialysis against water for two hours, the pH of a 1% solution rose to 5.5, which is the same as that given by the native protein.

Results and Discussion

Figure 1 gives the results of the viscosity and sedimentation measurements. The value of $[\eta]$ is 0.036 dl. g.$^{-1}$ in aqueous acetate buffer and 0.134 dl. g.$^{-1}$ in GAA. The sedimentation constants ($S_{20,w}$) in the same two solvents are 4.4 and 2.3 S, respectively. In order to determine how important electrostatic charge effects might be in

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