Formation Constants of Silver(I) Complexes of Some Sulphur-containing Dipeptides and Valylvaline

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Formation constants at 25 °C and I = 0.10 mol dm^{-3} (KNO_3) have been determined for the complexes of Ag^I with a range of nine dipeptides which incorporate side-chains containing one (glycylmethionine and methionylglycine) or two sulphur donor atoms. In the latter case dipeptides formed from amino acids of the same and of different chiralities were studied (*e.g.* L-methionyl-L-methionine and L-methionyl-D-methionine). The results are compared with those for valylvaline. Values for the formation constants are interpreted in terms of the preferred conformations of the dipeptides, and the tendency for Ag^I to bond to S-donor atoms or to adopt linear co-ordination through the formation of dimeric complexes.

The co-ordination sites of silver(I) ions with sulphur-containing dipeptides are not well defined, previous work on the subject being sparse and often contradictory. Early potentiometric studies of the acid-dissociation constants of several sulphur-containing amino acids with Ag^I were interpreted as suggesting that, in solution, co-ordination was *via* N(amino) alone, with little chelation through the thioether sulphur or the carboxylate oxygen atoms.[1,2] The authors reasoned that, since the formation constants were of the same magnitude as with aliphatic amino acids, $Ag{-}NH_2$ interactions must predominate. Other authors have disagreed with this. From spectrophotometric measurements, McAuliffe *et al.*[3] concluded that with Met † co-ordination was *via* the sulphur atom only. An n.m.r. study by Natusch and Porter [4] was also interpreted as indicating that co-ordination in both acidic and basic solutions was, in general, entirely S-co-ordination. The authors did, however, suggest that with smc chelation *via* sulphur and the amino-group was a possibility. Kozlowski and co-workers [5] were the first to carry out a study with dipeptides. They used ^1H n.m.r. spectroscopy to observe changes in bonding sites as a function of pH in silver(I) complexes of Met–Gly and Gly–Met. In acidic solutions the co-ordination site was the thioether sulphur alone, while in basic solution bonding took place *via* both nitrogen and sulphur. Their results suggested that, in basic solution, a mixture of $S{-}Ag{-}S$ and $S{-}Ag{-}N$ bonding was present.

Crystallographic studies have been performed only on Gly and Gly-Gly complexes of Ag^I.[6] They have shown the metal ion to have a characteristic linear two-fold geometry. Solid-state complexes are often co-ordinated *via* the carboxylate oxygen atom also. Such bonding is not detected in solution.[1,5,7]

The silver(I) ion shows a pronounced tendency to exhibit linear co-ordination. However, with sulphur-containing ligands, three- and four-co-ordinate species are often found. Potentiometric studies have shown that the enhanced stability of $Ag{-}S$ complexes is due to polarization of the ligand, rather than π bonding.[8] Silver(I), being a typically 'soft' acceptor, would be expected to bond to the soft base sulphur, and possibly to N(amino) rather than to oxygen.

We report here the results of a study of the complexes of Ag^I with a range of related dipeptides containing thioether donor centres. Two groups of ligands were considered. One group contained only one sulphur side-chain while the other contained two such side-chains, and included ligands with amino-acid residues with the same (*e.g.* LL) and different (*e.g.* LD) chirality. The results are compared with those for complexes of Ag^I with Val-Val.

† The following abbreviations are used throughout: Gly = glycine; Val = valine; Met = methionine; and smc = *S*-methylcysteine. Unless otherwise specified, all amino-acid residues are assumed to have the L configuration.

Experimental

Organic Syntheses.—The following ligands were synthesized: Val-Val, Val-D-Val, Gly-Met, Met-Gly, Met-Met, Met-D-Met, Met-smc, D-Met-smc, smc-Met, smc-D-Met, and smc-smc. Standard liquid-phase methods were used for all the syntheses.[9] The starting materials were optically pure amino acids (Sigma Chemicals Co.). The amino-groups were protected by synthesizing the *N*-t-butoxycarbonyl derivatives and the amino-acid residues were coupled using an active-ester method. This had the advantage of making C-protection of the second amino acid unnecessary.[10]

The active ester was prepared using *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodi-imide. Succinimide ester derivatives were recrystallized from isopropyl alcohol. The N-protected active esters were then coupled with the second amino acid in aqueous alkali ($NaHCO_3$ in a 1 : 1 tetrahydrofuran–water mixture) to give clear yellow oils. The t-butoxycarbonyl protecting group was removed at once using cold trifluoroacetic acid to give the trifluoroacetate. The free dipeptide was prepared from this by treatment with aqueous ammonia followed by recrystallization from a water–methanol mixture.

Purity was checked by t.l.c. and elemental analysis. The results are given in Table 1.

Potentiometric Studies.—Proton complex-formation constants were calculated from titrations of the ligands with alkali, changes in pH being followed with a glass electrode calibrated in terms of hydrogen-ion concentrations. Silver complex-formation constants were calculated from similar titrations in the presence of silver ions but, in this case, both hydrogen-ion and silver-ion concentrations were monitored using a glass electrode and a silver–silver chloride indicator electrode respectively,[11] both calibrated in terms of concentrations.[12] Perchloric acid (0.001 mol dm^{-3} in 0.10 mol dm^{-3} KNO_3) was used as a standard for hydrogen-ion concentrations and the precision of potentials was 0.1 mV (0.002 pH). The potentials of the electrodes were measured, relative to a saturated mercury(II) sulphate reference electrode, linked through a potassium sulphate salt bridge. Concentrations used were: ligand, 0.003; and silver, 0.0015 and 0.003 mol

Table 1. Preparative details for the dipeptides studied

Dipeptide	Found (%) C	H	N	S	Calc. (%) C	H	N	S	α */°
Val-L-Val	55.4	9.1	12.4		55.55	9.25	12.95		−17.5
Val-D-Val	55.45	9.35	12.8		55.55	9.25	12.95		+55.8
Met-Gly·0.25MeOH	40.1	6.7	13.1	15.1	40.65	7.0	13.1	15.0	+41.5
Met-L-Met	43.2	7.25	9.95		42.9	7.1	10.0		+7.5
Met-D-Met	42.5	7.05	9.9		42.9	7.1	10.0		+73.3
Met-smc	40.6	6.7	10.5		40.6	6.8	10.5		0
D-Met-smc	40.55	6.8	10.5		40.6	6.8	10.5		−87.8
smc-Met	40.45	6.75	10.5	23.9	40.6	6.8	10.5	24.1	−6.4
smc-D-Met	40.85	6.7	10.8	23.8	40.6	6.8	10.5	24.1	+67.3
smc-smc	37.85	6.35	11.0	25.4	38.1	6.35	11.1	25.4	−12.9

* At 293 K, 589 nm, in dioxane.

Table 2. Proton complex-formation constants (standard deviations 0.01) at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

Ligand	log K_{HL}	log β_{H_2L}	log K_{H_2L}
L-Val-L-Val	7.97	11.36	3.39
L-Val-D-Val	8.22	11.31	3.04
Gly-L-Met	8.22 [a]	11.33	3.11
L-Met-Gly	7.56 [b]	10.97	3.41
L-Met-L-Met	7.43	10.65	3.22
L-Met-D-Met	7.63	10.67	3.04
L-Met-smc	7.40	10.38	2.98
D-Met-smc	7.62	10.34	2.72
smc-L-Met	7.03	10.21	3.18
smc-D-Met	7.23	10.17	2.94
smc-smc	7.03	9.95	2.92

[a] 8.19.[15] [b] 7.56.[15]

dm^{-3}. The ionic strength of all solutions was adjusted to 0.10 mol dm^{-3} with KNO_3.

Formation constants were calculated using the computer program MINIQUAD,[13] which can handle 'two-electrode' titrations. The additional data provided by the silver–silver chloride electrode were necessary because the Ag^I–sulphur-containing dipeptide systems are much more complicated than, for example, Cu^{II}–dipeptide systems, This is a result of extensive polynuclear and protonated-complex formation. What is more, Ag–S co-ordination will only cause proton dissociation by a secondary effect since a thioether sulphur does not normally protonate. Hence the necessity for more experimental data if reliable results are to be obtained.

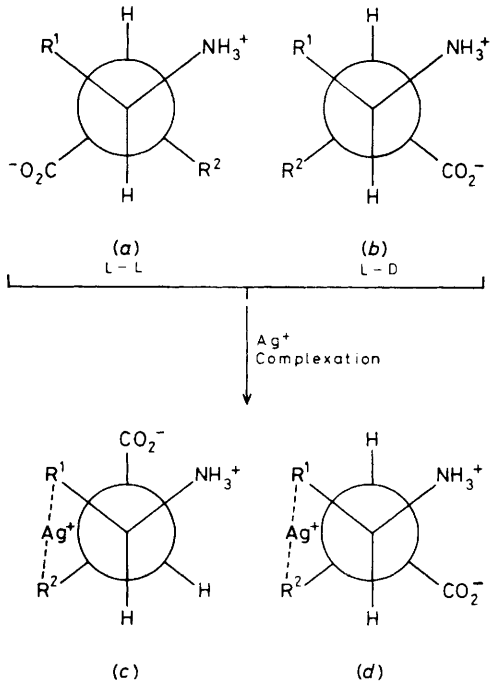


Figure 1. Conformations of neutral (zwitterionic) dipeptides: (*a*) amino acids of same chirality (preferred conformation not charge stabilized); (*b*) amino acids of opposite chirality (charge-stabilized β conformation); (*c*) the LL-dipeptide must change conformation to form the [AgHL] complex; and (*d*) the LD-dipeptide can retain the β conformation in the [AgHL] complex

Results and Discussion

Proton Complex Formation.—Proton complex-formation constants for the ligands studied are given in Table 2, where K_{HL} refers to protonation of the amine group and K_{H_2L} to carboxylate protonation. The values are in good agreement with literature values for dipeptides containing glycine.[14,15] Stereoselective effects between diastereoisomeric pairs are significant, and in all cases result from an enhanced stability of the zwitterionic form of the racemic ligand. Hence log K_{HL} is always higher for the ligands containing amino-acid residues of different chirality (*e.g.* LD) while log K_{H_2L} is lower, demonstrating the larger pH range of existence of the HL species. Values for the overall formation constants (log β_{H_2L}) show little stereoselectivity. These results are in good agreement with trends reported previously [16] and result from the favourable folding of the (LD) dipeptide in the β conformation as demonstrated in Figure 1.

Silver Complex Formation.—It was found possible to obtain a reliable fit without significant drift between measured and calculated values for the electrode potentials between pH 3 and 8.5. To achieve this, it was necessary to include both monomeric and dimeric complex species together with the bis complex [AgL_2]. Calculated values for the formation constants are given in Table 3 where figures in parentheses are standard deviations and make no allowance for systematic errors. They do, however, give a good indication of the importance of the species concerned, major species generally showing small standard deviations.[13] The Scheme accounts for all the species formed (HL = neutral amino acid).

In acidic solution the important species are the protonated complexes [AgH_2L] and [AgHL] (or, to a lesser extent, its dimer [$Ag_2H_2L_2$]) (charges omitted for clarity). Figure 2 is a graph of $-E_{Ag}$ *vs.* pH for all the systems studied and repre-

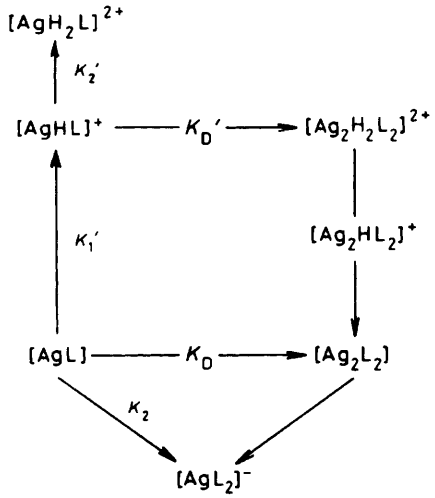
Table 3. Silver complex-formation constants (standard deviations given in parentheses) for the species $Ag_xH_yL_z$ at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

	log β_{xyz}						
Ligand	101	102	111	121	202	212	222
L-Val-L-Val	3.03(5)	6.93(5)					
L-Val-D-Val	3.24(5)	7.23(4)					
Gly-L-Met	4.70(1)	8.03(4)	11.91(1)	17.4(1)	12.34(1)	19.49(3)	26.70(2)
L-Met-Gly	4.63(2)	8.29(2)	10.72(1)	15.47(1)	12.45(1)	18.70(1)	24.53(2)
L-Met-L-Met	5.81(1)	8.7(1)	11.86(1)	16.31(7)	14.44(1)	20.88(1)	26.78(2)
L-Met-D-Met	5.71(2)	?	12.40(1)	17.3(2)	14.51(1)	21.17(2)	27.76(4)
L-Met-smc	5.72(1)	8.1(2)	12.05(1)	16.61(8)	14.19(1)	20.84(1)	26.83(2)
D-Met-smc	5.77(1)	8.6(1)	12.40(1)	17.1(1)	14.45(1)	21.07(3)	27.74(2)
smc-L-Met	6.01(1)	9.66(7)	11.02(1)	14.55(5)	14.81(1)	20.19(1)	24.81(2)
smc-D-Met	5.86(1)	9.40(2)	11.76(1)	15.51(6)	14.74(1)	20.66(1)	26.45(1)
smc-smc	6.19(1)	9.59(1)	11.53(1)	14.75(3)	15.13(1)	20.79(1)	25.82(1)

Stepwise and derived constants (log values) *

	K_2	K_1'	K_2'	K_1''	K_D	K_D'
L-Val-L-Val	3.9					
L-Val-D-Val	4.0					
Gly-L-Met	3.3	7.21	5.5	3.69	2.9	2.9
L-Met-Gly	3.7	6.09	4.75	3.16	3.2	3.1
L-Met-L-Met	3.0	6.05	4.45	4.42	2.8	3.1
L-Met-D-Met	?	6.69	4.9	4.77	3.1	3.0
L-Met-smc	2.5	6.33	4.56	4.65	2.8	2.7
D-Met-smc	2.8	6.63	4.7	4.78	2.9	2.9
smc-L-Met	3.6	5.01	3.53	3.99	2.8	2.9
smc-D-Met	3.5	5.90	3.73	4.53	3.0	2.9
smc-smc	3.4	5.34	3.22	4.50	2.8	2.8

* K_2 = $[AgL_2]/[AgL][L]$, K_1' = $[AgHL]/[AgL][H]$, K_2' = $[AgH_2L]/[AgHL][H]$, K_1'' = $[AgHL]/[Ag][HL]$, K_D = $[Ag_2L_2]/[AgL]^2$, and K_D' = $[Ag_2H_2L_2]/[AgHL]^2$.



Scheme.

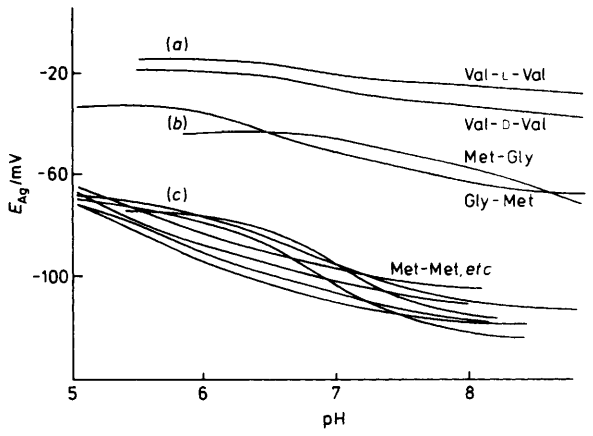


Figure 2. Relationship between $-E_{Ag}$ and pH during titrations of dipeptides in the presence of Ag^+ (Ag : L = 1 : 1) containing none (*a*), one (*b*), or two (*c*) sulphur donor atoms

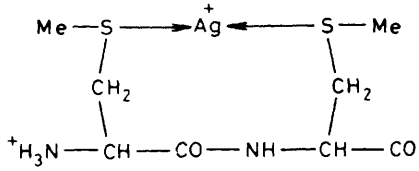
sents the amount of silver participation in complexation at any given pH. In acidic solution (up to pH 5) addition of alkali has a negligible effect on silver concentration, simply ionizing the proton from the non-co-ordinating carboxylate group. Above pH 5, with sulphur-containing dipeptides there is a sharp change in $-E_{Ag}$, and the important species in basic solution are [AgL] (or its dimer) and $[AgL_2]$. It was often found that the dimer, $[Ag_2L_2]$, remained in the computer model in preference to [AgL] and the importance of the dimers was confirmed by repeating the titrations at different concentrations.

One particularly striking feature of Figure 2 is the difference in the shapes of the curves for families of dipeptides containing (*a*) no sulphur donor atoms, *e.g.* Val-Val, (*b*) only one sulphur donor, *e.g.* Met-Gly, and (*c*) two sulphur atoms, *e.g.* Met-Met. From Figure 2 and the Nernst equation it is clear that the order of stability of the complexes is (*c*) ≫ (*b*) ≫ (*a*) suggesting different modes of complexation for the three families.

Since Val-Val was the simplest dipeptide studied it may be used as a comparison for the other systems. The aliphatic side-chains of Val-Val do not contain co-ordination sites leaving only N(amino), N(peptide), and O(carboxylate) as

potential donor centres. No evidence has been found to show significant co-ordination in solution of Ag^+ to N(peptide) or O(carboxylate),[1,5] making N(amino) the only significant donor.[7] The only complexes formed with Val-Val were [AgL] and [AgL_2] and the formation constants were compatible with other silver–ammine interactions.[6,14] The stepwise formation constant for [AgL_2] was log $K = 3.90$, larger than log K_{AgL} (3.03), in keeping with the preference of Ag^+ for two-fold linear co-ordination. The stereoselectivity was small but significant (0.2 long units) and in favour of complexes with Val-D-Val. This stereoselectivity was reflected almost entirely in values for K_{AgL}.

The formation constants and structures for the remaining families of complexes cannot be explained so easily. For all these systems the first complexes formed in acid solution are protonated species in which co-ordination must take place *via* thioether sulphur only.[3-5] Table 3 lists the stepwise constants for the addition of Ag^+ ions to fully and partially protonated dipeptide ligands. Comparison of the values for ligands with only one sulphur atom shows greater stability for complexes with Gly-Met than with Met-Gly. This is the result of steric interference with a protonated functional group, which has a greater destabilizing influence on the amino-group than the carboxylate group. Thus it is clear that Ag^I prefers to co-ordinate to the thioether sulphur when it is in the carboxylate terminal of the dipeptide. Values for log K_{AgHL} ($Ag + HL \rightleftharpoons AgHL$) for dipeptides containing two sulphur atoms are considerably greater than for Met-Gly and Gly-Met, compatible with linear co-ordination of silver to both sulphur atoms of the dipeptide. Hence the structure of [AgHL] complexes with these ligands will be as shown below. The dimeric species can be envisaged as co-ordinating to silver *via* the thioether atoms of the two dipeptide molecules.



Significant stereoselective effects are apparent from Table 3 showing that for both stepwise constants the LD isomers are relatively more stable than the LL analogues. Conformational analysis of the free protonated dipeptides and of their silver complexes shows that while the monoprotonated LD-dipeptides can retain their preferred β conformation on complex formation through the sulphur atoms, the LL dipeptides have to undergo a conformational change to a more sterically hindered form which will tend to destabilize the silver complexes relative to those of the LD isomers. This is demonstrated in Figure 1. The data in Table 3 also suggest that the diprotonated analogues, [AgH_2L], have greater stabilities than the monoprotonated analogues. This suggests that the presence of a proton on the carboxylate group is a stabilizing influence on the Ag–S bonds. The largest effect is with Gly-Met where silver is adjacent to the carboxylate group.

In basic solution the major species are [AgL] (or its dimer) and the bis complex [AgL_2]. These could involve co-ordination through both sulphur and nitrogen donors, or through the sulphur only. Formation constants for these complexes fall into the three main groups identified in Figure 2. Complexes with Gly-Met and Met-Gly are significantly more stable than with Val-Val, while complexes with ligands containing two sulphur donors are significantly more stable still.

Considering the second group, if co-ordination were through both N(amino) and S(thioether), Gly-Met and Met-Gly would be expected to have significantly different formation constants as a result of the different chelate ring sizes. No such difference is observed suggesting that the bonding is essentially the same. Hence, even when $^-NH_3^+$ is deprotonated, co-ordination takes place only through the thioether sulphur atom. With the third group bonding must take place through both sulphur atoms. Usually, linear co-ordination of Ag^I cannot take place on chelate formation, but with dipeptides the sulphur atoms are situated far enough apart to minimize ring strain and make chelation possible with linear co-ordination and a large chelate ring.

The second stepwise constants (formation of [AgL_2]) reveal a reduced tendency, relative to Val-Val, for the mono complexes of sulphur-containing dipeptides to co-ordinate a second ligand. Among the ligands with two sulphur atoms it is noticeable that log K_{AgL} for Met-sms is smaller than for sms-Met complexes.

The dimeric species, [Ag_2L_2], are important in all these systems, often being retained in the computer model to the exclusion of the monomer. Values for log K_D are shown in Table 3. The dimers, containing only Ag–S co-ordination, allow linear co-ordination but form very large chelate rings (up to 18-membered). While such large rings may seem unlikely, they will be stabilized by electrostatic interaction between the functional groups. Dimer formation with Gly-Met and Met-Gly must involve both Ag–S and Ag–N co-ordination. In mildly acidic solution an intermediate dimeric species, [Ag_2HL_2] is also present.

One interesting feature is the change in protonation constants of the silver complexes compared to the free dipeptides. Values for the dipeptides studied are given in Table 3. These show clearly that co-ordination of silver favours protonation of the carboxylate group, and deprotonation of the amine nitrogen, greatly decreasing the range of existence of the zwitterionic species. The reason for the enhanced stability of a protonated carboxylate is not immediately apparent.

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