Association behaviour of selected amino acid and oligopeptide derivatives with fluorinated alcohols

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The interaction behaviour of different amino acid, dipeptide and tripeptide derivatives with fluorinated proton donor molecules has been studied by IR spectroscopy. It is shown that the strength of the association depends on the steric dimensions of the residues in the side chain.

Introduction

As we have shown recently,[1] amino acid derivatives form intramolecular hydrogen bonds between NH and the ester carbonyl functionalities. These so-called C_5 rings were only observed in non-polar media such as hexane and carbon tetrachloride. Similar intramolecular associations of the C_7 type have also been observed in several dipeptides.[2] An exception to this effect occurs in Z-L-Phe-L-Val-OMe, wherein the phenyl ring of the side chain sterically shields the urethane group (Z = benzyloxycarbonyl). The fluorinated alcohols used in our experiments are often used as solvents for the investigation of the circular dichroism of proteins. From these measurements[3] it is known that, in comparision to measurements in chloroform, 1,1,1,3,3,3-hexafluoropropan-2-ol (HFiP) may change the helicity of the protein. This alcohol, being a strong proton donor, shows a conformational equilibrium of *trans/gauche* rotamers, resulting from rotation on the C–O bond axis, which is clearly observable in its IR spectrum.[4] Therefore, in the context of our work, the protonation potency of these proton donors as well as the influence of their conformational heterogeneity was sufficiently interesting for us to start the investigations described in this paper.

Experimental

Substances

1,1,1,3,3,3-Hexafluoropropan-2-ol (HFiP) obtained from Merck was used without further purification. (*R*)-(+)-2,2,2-Trifluoro-1-phenylethanol (TFPE) was distilled and stored over molecular sieves. The optical rotation of the pure liquid was +40.

The amino acid Z-L-Val-OMe and Z-L-Phe-OMe, dipeptide Z-L-Phe-L-Val-OMe and tripeptide derivatives Z-L-Phe-L-Ala-L-Val-OMe were prepared in the organic institute of our department by H. Jeschkeit; their physical characteristics are described elsewhere.[1,2] The designation follows the usual abbreviation recommended by the IUPAC for nomenclature of biopolymers.

As the solvent we used carbon tetrachloride which was stored over molecular sieves. The concentration of the alcohol was limited by its self-association and did not exceed 1.5×10^{-2} mol dm^{-3}. Peptide concentrations were in general between 1.8×10^{-3} and 8.6×10^{-3} mol dm^{-3} in order to be comparable with former studies of these compounds.[2]

Measurements

The IR spectra were recorded on a Bruker IFS 25 spectrometer equipped with a self-built temperature device based on Peltier elements. The thickness of the NaCl cells was *ca.* 3 mm except in a few cases where 0.5 mm cells were used. The spectra were recorded at −10, 25 and 40 °C with a resolution of 2 cm^{-1}.

Software

By use of OPUS 2.0 (Bruker) the CO and NH region of the spectra were baseline corrected and with the help of a band deconvolution program 'Peakfit-program for non-linear curve fitting' (Jandel Scientific) the band profiles were simulated as a set of Pearson functions. With the help of this program we were able to simulate the band profiles with an accuracy of >99.9%. The number of signals and the position of their maxima were determined from the difference spectra of the band profile at −10 and 40 °C and also from the first derivative of the band structure.

Calculations of the equilibrium constants were based on the change of the OH or of the separated CO band intensities depending on which signal was better resolved. The details of the method are described in ref. 5.

ΔH Values were calculated from the temperature dependence of the equilibrium constants according to the van't Hoff equation.

Results and discussion

Qualitative aspects

The OH–CH region of the solutions of the pure alcohol (*a*), the pure Z-L-Val-OMe (*b*), the mixture of the alcohol solution and the Z-L-Val-OMe solution (*c*) as well as the difference spectra (*d*) are shown in Fig. 1. As can be seen from Fig. 1, the intensity of the OH doublet due to the *gauche* and *trans* conformers of HFiP decreases upon addition of the amino acid derivative. Simultaneously, a broad OH association band was observed overlapping the NH signals of the Z-L-Val-OMe. In the band contour of the difference spectra (*d*) there exists an absorption

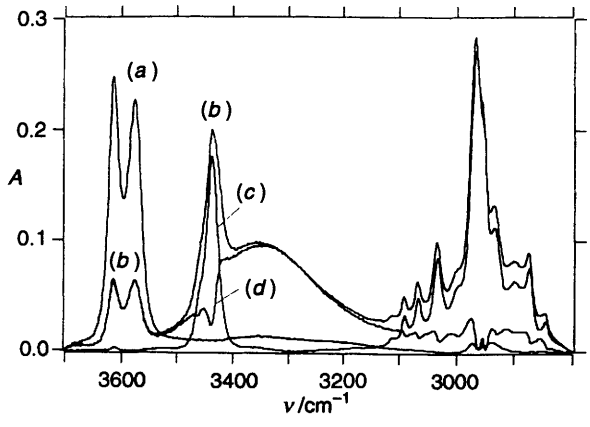

Fig. 1 OH–NH region showing the association of Z-L-Val-OMe and HFiP in CCl_4 at −10 °C: (*a*) HFiP solution, 8.10×10^{-3} mol dm^{-3}; (*b*) HFiP solution, Z-L-Val-OMe added, 5.28×10^{-3} mol dm^{-3}; (*c*) Z-L-Val-OMe solution, 4.93×10^{-3} mol dm^{-3}; (*d*) difference spectrum between (*b*) and (*c*)

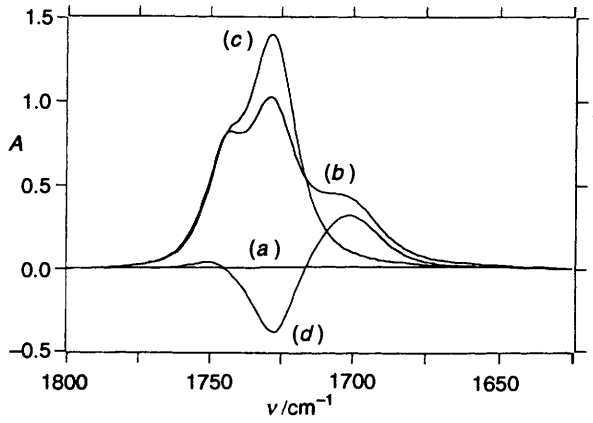

Fig. 2 CO region showing the association of Z-L-Val-OMe and HFiP (see Fig. 1 for details)

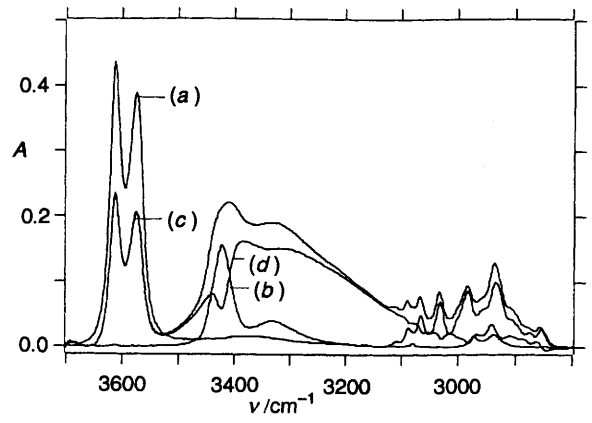

Fig. 3 Interaction of HFiP and Z-L-Phe-L-Ala-L-Val-OMe in CCl_4 at −5 °C: (*a*) HFiP solution, 9.91 × 10^{-3} mol dm^{-3}; (*b*) peptide solution, 2.09 × 10^{-3} mol dm^{-3}; (*c*) HFiP–peptide solution, at same concentrations; (*d*) difference of (*c*) and (*b*)

Table 1 Position of the associated CO bands during the association of the protected amino acids with different alcohols dissolved in CCl_4

System	$\nu(CO_{ass})/cm^{-1}$	$\Delta\nu/cm^{-1}$
HFiP–Z-L-Val-OMe	1702	28
TFPE–Z-L-Val-OMe	1706	24
TFPE–Z-L-Phe-OMe	1706	20

Table 2 Positions of the associated CO signals (cm^{-1}) of the interaction of HFiP and Z-L-Phe-L-Val-OMe in CCl_4

	Urethane		Peptide	
System	$\nu(CO_{ass})$	$\Delta\nu$	$\nu(CO_{ass})$	$\Delta\nu$
HFiP–Z-L-Phe-L-Val-OMe	1705	20	1662	23

gap in the NH region, which shows that the compensation of the NH signal does not succeed completely. The reason for this is the slight shift of the NH bands of the alcohol–amino acid derivative solution to lower wavenumbers. This effect can be understood when we inspect the corresponding CO region, which is given for the system HFiP–Z-L-Val-OMe in Fig. 2. The precise positions of the three absorption maxima of the associated CO(urethane)-groups in the systems HFiP–Z-L-Val-OMe, TFPE/Z-L-Val-OMe and TFPE–Z-L-Phe-OMe after band deconvolution are given in Table 1.

In ref. 2 the intense signal at 1730 cm^{-1} was interpreted as a vibration of the urethane function. The shoulder frequency is due to the ester carbonyl function. Besides these two bands an additional signal at lower frequency was found when the alcohol was added. With an increase in temperature, the intensity of this signal strongly decreased, while the intensity of the urethane CO function increased significantly. No intensity change of the ester CO absorption was observed. Therefore, we conclude that the interaction of the alcohol molecules occurs only at the urethane group. The ester CO is not affected and thus the formation of C_5 rings, in which this group is involved is also not affected. However, the interaction at the urethane group may influence the properties of the NH function in the immediate vicinity. This function should become more acidic and is shifted to higher wavenumbers. This effect is the reason that the bands of the free NH groups cannot be compensated completely, as we have mentioned above.

Huyskens *et al.*[6] observed the partial disruption of the C_5 intramolecular associates in the *N-tert*-butoxycarbonylsarcosin-*N*,*N'*-dimethylamide as a consequence of an interaction with phenols. This difference from our results can be explained by considering the steric effects of the *tert*-butyl group, which hinders the interaction with the urethane functionality.

Dipeptide derivative

Z-L-Val-OMe contains one additional acceptor function which is involved in a C_5 ring. Therefore, we expected a different association behaviour according to the basicity of the urethane, peptide and ester CO functions. The NH region of the spectrum is overlapped by the intense and broad absorption of the associated OH band. Its maximum is located at 3300 cm^{-1}. The CO region shows two additional signals in comparison to the spectra of the pure dipeptide solution. The maxima of the separated CO bands are summarized in Table 2.

Tripeptide derivative

In contrast to Z-L-Ala-L-Phe-L-Val-OMe[2] the tripeptide Z-L-Phe-L-Ala-L-Val-OMe, in which only the sequence of the amino acids is changed, does not form C_7 associate rings in carbon tetrachloride. The CO region of this substance is therefore only presented by the absorptions of the free CO groups (if the C_5 rings are neglected). The Amide I absorption of the peptide groups in Z-L-Phe-L-Ala-L-Val-OMe occurs as a doublet due to conformational heterogeneity caused by the steric hindrance of the free rotation of the side chain residues.

When the fluorinated alcohol is added, the same effect in the NH region has been found as we have discussed for the interaction of amino acid and peptide derivatives (Fig. 3).

The CO region of this solution is different from that of the pure peptide solution (Fig. 4). At −5 °C the intensity of the ester CO signal is evidently smaller. Contrary to the cases discussed above, donor molecules also interact with this function.

Analysis of the corresponding region for the bending vibration of the C–O–C function, which is assumed to be near 1200 cm^{-1}, indicates that an interaction at this position cannot be excluded. By decreasing the cell thickness from 3 to 0.5 mm and, therefore, allowing the necessary increase of the concentration, we observed an additional absorption at 1170 cm^{-1}. The formation of this signal seems to be proportional to the decrease of the intensity of the absorption at 1200 cm^{-1} and should be, therefore, an indicator for the change of the vibration modes due to the self-association. Upon addition of more alcohol molecules into the peptide solution, the signal at 1200 cm^{-1} does not occur even at higher temperatures. Contrary to the discussion given above, the absorptions of the associated structure are shifted to lower wavenumbers. Therefore an

Table 3 Equilibrium constants, ΔH and ΔS for the alcohol–amino acid interaction in CCl_4

System	K_1 (−10 °C)	K_2 (25 °C)	K_3 (40 °C)	ΔH/kJ mol^{-1}	ΔS/J mol K
HFiP–Z-L-Val-OMe	183	71	55	−16.8	−20.6
TFPE–Z-L-Val-OMe	96	51	46	−10.4	−1.7
TFPE–Z-L-Phe-OMe	74	31	24	−15.3	−22.6

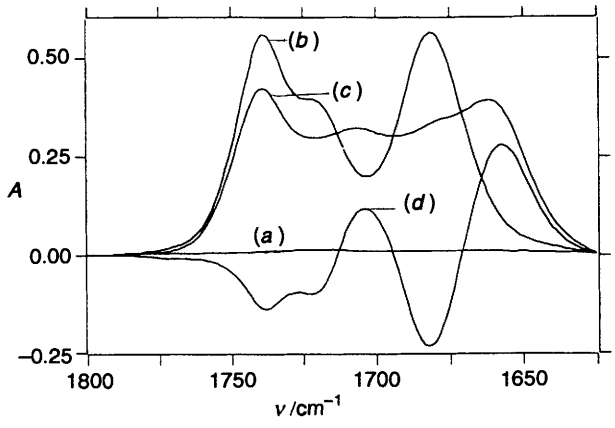

Fig. 4 Interaction of HFiP and Z-L-Phe-L-Ala-L-Val-OMe (see Fig. 3 for details)

association of the alcohol OH group at the ester CO function seems probable. This phenomenon was not found in the case of the dipeptide Z-L-Phe-L-Val-OMe or in the protected amino acids.

Quantitative effects

Thermodynamic parameters of the association of HFiP–protected amino acids. As the interaction of HFiP and TFPE only occurs at one carbonyl function at the urethane group, the equilibrium constant can be calculated from the decrease in the intensity of the OH and CO absorptions. In Table 3 the equilibrium constants, ΔH and ΔS values for the interaction of HFiP and TFPE with Z-L-Phe-OMe and Z-L-Val-OMe are given.

In comparison to other acceptor molecules like acetone and tetrahydrofuran, the amino acids behave like weaker acceptor molecules.[4] Both alcohols should be comparable in their acidity because the substitution of one CF_3 group of HFiP by a phenyl system results in the TFPE molecule. Similar conclusions can be derived from the frequencies of the OH groups, which were found in methylene chloride at 3584 and 3590 cm^{-1} for HFiP and TFPE, respectively. Therefore the differences in the thermodynamic data for the systems HFiP-Z-L-Val-OMe and TFPE–Z-L-Val-OMe should result from steric hindrance. In particular, the low value of entropy change in the case of the system TFPE–Z-Val-OMe may be due to the low reaction enthalpy, which itself may result from the limited approach of the interacting proton to the basic centre, due to steric bulkiness of the trifluoromethylphenyl arrangement.

Interaction of dipeptide Z-L-Phe-L-Val-OMe with HFiP. Based on the band deconvolution of the overlapping CO signals, the equilibrium constants for both acceptor positions of the dipeptide derivative Z-L-Phe-L-Val-OMe can be calculated. The K values at different temperatures for the association of HFiP and Z-Phe-Val-OMe are summarized in Table 4. The values of the interaction at the urethane group are only slightly higher than those in Table 3. Differences in the association behaviour are reflected by the temperature dependence of the

Table 4 Equilibrium constants of the interaction with different CO groups in the systems formed by HFiP and Z-L-Phe-L-Val-OMe in CCl_4

Acceptor position	K_1 (−10 °C)	K_2 (25 °C)	K_3 (40 °C)
Urethane CO	212	92	80
Peptide CO	178	49	23

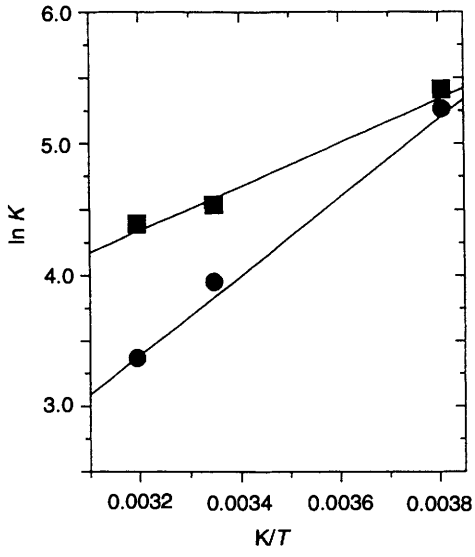

Fig. 5 ln K *vs.* $1/T$ plot presenting the association on both acceptor positions of Z-L-Phe-L-Val-OMe with HFiP in CCl_4: (■) urethane CO; (●) peptide CO

equilibrium constants. This is graphically demonstrated in the ln$K$ *vs.* $1/T$ diagram (Fig. 5).

Excluding the mutual influence of both equilibria, the ΔH values were approximated from the slope of the curves and were found to be −14 and −25 kJ mol^{-1} for the interaction at the urethane and the peptide CO function, respectively.

At temperatures of 25 and 40 °C the dominant amount of molecules may be assumed to be linearly arranged considering the high number of C_5 associated structures. The probability to interact with donor molecules is, therefore, limited by the rotation about the C^{α}–C^{β} bond axis and consequently by the steric requirements of the side chains. The phenyl system of the Phe residue should theoretically be able to protect both acceptor functions. The relatively large K values and their small temperature variation lead to the conclusion that the phenyl group inhibits association to a large extent.

Interaction of the tripeptide Z-L-Phe-L-Ala-L-Val-OMe. The results obtained by using the deconvoluted CO area in order to calculate the equilibrium constants for the association on the ester, peptide and urethane CO groups are given in Table 5. Although the temperature dependence of the equilibrium constants is lower than for the dipeptide, in the main similar tendencies were found. Accordingly, due to the smaller acidity of the ester CO function the association with this group is weaker than with the amide functions. The two peptide functions existing in tripeptides cannot be resolved by means of our

Table 5 Equilibrium constants and ΔH values for the association of HFiP with different acceptor positions of Z-L-Phe-L-Ala-L-Val-OMe

Acceptor position	K_1 (−10 °C)	K_2 (25 °C)	K_3 (40 °C)	ΔH/kJ mol^{-1}
Peptide	138.8	54.3	38.1	−19.9
Urethane	126.5	60.5	43.8	−16.4
Ester	41.9	13.2	9.6	−22.7

deconvolution program, but in fact the corresponding band represents two CO groups.

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