Characterization of helical sense by infra-red measurement

H. Yamamoto, K. Inouye and T. Hayakawa

Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda 386, Japan

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Infra-red spectroscopy is now established as one of the most important and simple methods for the elucidation of polymer structures. Polypeptides or proteins exhibit characteristic infra-red bands at about 1650 cm^{-1} (amide I) and 1550 cm^{-1} (amide II). The dependences of these amide I and II bands upon the chain conformations have been found and these bands have been used for structure diagnoses of polypeptide chain conformations. Miyazawa *et al* [1] have calculated that the left-handed helix of polypeptides should show the amide I band 8 cm^{-1}, and the amide II band 2 cm^{-1}, to higher frequencies of the right-handed helix. The theoretical calculation has been borne out by experiment both in solution and in the solid states using poly(L-aspartates)[2,3]: left-handed poly(L-aspartates) such as β-methyl and β-benzyl show the amide I band at 1666–1668 cm^{-1} and the amide II at 1557 cm^{-1} in chloroform or in films, and right-handed poly(L-aspartates) such as β-ethyl, β-n-propyl, β-isopropyl, β-n-butyl and β-phenethyl show the amide bands at 1656–1659 and 1553 cm^{-1} in the same condition.

Since the comparison of infra-red spectra has, until now, not been reported using poly(L-amino-acids) and poly(D-amino-acids) with exactly the same side chains, we widened the experiments on the L-polypeptides to include a number of D-homo- and copolypeptides. In the present Note we prepared 30 or more helical L- and D- homo and copolypeptides and compared their amide band frequencies. The polypeptide samples listed in *Table 1* were all prepared by the *N*-carboxyanhydride method[4]. The intrinsic viscosities of the polypeptides were in the range 0.38–1.05 dl/g in dichloroacetic acid at 25°C. The infra-red absorptions in the range 4000–650 cm^{-1} were measured on Jasco Model DS-301, IRA-1 and A-3 spectrophotometers. For the infra-red measurements, the film samples of the polypeptides were cast from the solution in chloroform or *m*-cresol.

The characteristic amide I and II bands of the helical polypeptides are listed in *Table 1* including the DL-polypeptides and the previous references. The results obtained have led us to the following conclusions. The typically α-helical poly(L-amino-acids) such as poly[Glu(OBzl)], poly(Met) and poly[Tyr(Z)] and their optical antipode poly(D-amino-acids) with exactly the same side chains give exactly the same amide I and II frequencies. Then as a general rule we could postulate that a polypeptide from L-amino-acid takes inherently a right-handed helix. The right-handed poly(L-amino-acids) such as poly[Glu(OBzl)], poly(Met), poly[Tyr(Z)] and poly[Asp(OEt)], which fit the above general rule, show the amide I at 1650–1659 cm^{-1} and the amide II at 1544–1548 cm^{-1}. In the special cases against the above rule the left-handed poly(L-amino-acids) such as poly[Asp(OMe)] and poly[Asp(OBzl)] show the higher amide bands at 1666–1668 and 1550 cm^{-1}. The left-handed helical poly(D-amino-acids) show the same frequencies (at 1650–1659 and 1544–1548 cm^{-1}). The opposite right-handed helical poly(D-amino-acids) give also the amide I band about 8 cm^{-1} and the amide II band about 2 cm^{-1} to higher frequencies of the left-handed helix. Similar changes of the bands have been observed for the copolymer series of benzyl aspartate with methyl or ethyl aspartate. Our experimental results fit perfectly with the normal coordinate calculations of Miyazawa *et al.*[1] who treated the infra-red active chain vibrations of the left-handed and right-handed α-helical forms of poly(L-

Table 1 Characteristic amide bands of polypeptides

Polypeptide	Helical sense	Amide I (cm^{-1})	Amide II (cm^{-1})	Reference
L-(or rich) amino-acid:				
Poly(Ala)	Right	1658	1548	1
Poly(Ala)	Left	1665	1550	1
Poly[Glu(OBzl)]	Right	1650	1546	15
Poly(Met)	Right	1650	1544	16
Poly[Tyr(Z)]	Right	1659	1546	
Poly[Asp(OMe)]	Left	1666	1550 (1557)[a]	3
Poly[Asp(OEt)]	Right	1659	1548 (1553)	3
Poly[Asp(OBzl)]	Left	1668	1550 (1557)	3
Poly[Asp(OBzl)^{90}Asp(OMe)^{10}][b]	Left	1666	1550	
Poly[Asp(OBzl)^{90}DAsp(OMe)^{10}]	Left	1667	1549	
Poly[Asp(OBzl)^{10}Asp(OMe)^{90}]	Left	1666	1550	
Poly[DAsp(OBzl)^{10}Asp(OMe)^{90}]	Left	1668	1550	
Poly[Asp(OBzl)^{90}Asp(OEt)^{10}]	Left	1665	1549	
Poly[Asp(OBzl)^{90}DAsp(OEt)^{10}]	Left	1667	1550	
Poly[Asp(OBzl)^{10}Asp(OEt)^{90}]	Right	1660	1548	
Poly[DAsp(OBzl)^{10}Asp(OEt)^{90}]	Right	1659	1547	
D-(or rich) amino-acid:				
Poly[DGlu(OBzl)]	Left	1650	1546	
Poly(DMet)	Left	1650	1544	
Poly[DTyr(Z)]	Left	1659	1546	
Poly[DAsp(OMe)]	Right	1666	1550	
Poly[DAsp(OEt)]	Left	1659	1548	
Poly[DAsp(OBzl)]	Right	1668	1550	
Poly[DAsp(OBzl)^{90}Asp(OMe)^{10}]	Right	1666	1549	
Poly[DAsp(OBzl)^{90}DAsp(OMe)]^{10}	Right	1667	1551	
Poly[Asp(OBzl)^{10}DAsp(OMe)^{90}]	Right	1664	1549	
Poly[DAsp(OBzl)^{10}DAsp(OMe)^{90}]	Right	1666	1550	
Poly[DAsp(OBzl)^{90}Asp(OEt)^{10}]	Right	1666	1549	
Poly[DAsp(OBzl)^{90}DAsp(OEt)^{10}]	Right	1664	1550	
Poly[Asp(OBzl)^{10}DAsp(OEt)^{90}]	Left	1661	1548	
Poly[DAsp(OBzl)^{10}DAsp(OEt)^{90}]	Left	1661	1548	
DL-amino-acid				
Poly[DLGlu(OBzl)]	Coil	1662	1555	
Poly(DLMet)	Coil	1657	1548	
Poly[DLTyr(Z)]	Coil	1663	1550	

[a] Values in parentheses were reported in ref 3; [b] Mol % of the starting *N*-carboxyanhydrides

alanine) for studying the effect of the sense of the helix.

It is therefore necessary to establish that the polypeptide is helical. The conformations determining whether the polypeptide is helical or not should be confirmed by other methods such as circular dichroism (c.d.) and nuclear magnetic resonance (n.m.r.) since helical poly(amino-acids) with opposite senses show, unfortunately, almost the same amide bands as found for the random coil polypeptides (at about 1660 and 1550 cm^{-1}).

C.d., n.m.r. and X-ray results can also give an indication of conformation. However, the c.d. spectra of aromatic polypeptides such as poly(Phe)[5], poly(Tyr)[6–8], poly(Trp)[9,10] and poly(Dopa)[11] are very anomalous and give little information about their helical sense since the $n-\pi^*$ peptide transition involves the contribution of the ^1L_a and ^1L_b transitions of the substituted benzenes in the Platt notation[12–14]. The n.m.r. spectrum is not always easy to measure in some solvents such as dioxane, dimethyl sulphoxide and trimethyl phosphate. X-ray analysis is elaborate and it is difficult to obtain information about the helical sense. The infra-red method has an advantage over c.d. in certain circumstances; that is, when the polypeptide has an aromatic side chain as described above and the c.d. spectrum of the polypeptide cannot be measured in the u.v. region owing to high absorption of the solvent. Because of the widespread use of infra-red techniques in the study of conformations of both polypeptides and proteins, we feel that the technique we have described here represents a facile method of characterization of the sense of helical conformation.

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Effect of concentration and temperature on the partial specific volume of a polystyrene sample in *trans*-decalin

B. Nyström and J. Roots

Institute of Physical Chemistry, University of Uppsala, Box 532, S-75 21 Uppsala, Sweden

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INTRODUCTION

The accuracy of molecular weight, frictional coefficient and other related data obtained from sedimentation velocity, diffusion and sedimentation equilibrium measurements on solutions of macromolecules is often restricted by a lack of accurate partial specific volume data, especially when studies are extended to higher concentrations. The partial specific volume, v_2, is not only a characteristic parameter of a certain macromolecule, it may also depend on solvent, concentration[1–5] and temperature[4,6,7].

Recently both the concentration and temperature dependence of the frictional coefficient obtained from sedimentation velocity measurements[8] were studied in wide ranges for the system polystyrene/*trans*-decalin[9]. In order to evaluate the frictional coefficient over the entire concentration and temperature interval in an unambiguous way, the concentration and temperature dependence of the partial specific volume of the solute was needed. Therefore specific volume measurements on solutions of a polystyrene sample in *trans*-decalin in the temperature range 20°–40°C and for mass fractions, w_2, ranging from 0.005 to 0.11 were performed.

Polystyrene in *trans*-decalin is a system with upper critical solution temperatures ranging from approximately 0° to 20°C; the actual temperature depending on the molecular weight of the sample[10]. From osmotic pressure measurements[11], it was observed that *trans*-decalin is a θ-solvent for polystyrene at 21°C (different θ-values have been reported in the temperature interval 20°–24°C[10,12–14]) and that it is considered to approach the good solvent region at about 40°C. Since the thermodynamic properties vary when going from θ- to good solvent conditions, it is plausible that this variation will affect the concentration dependence of the partial specific volume.

EXPERIMENTAL

A polystyrene sample with a narrow molecular weight distribution obtained from Pressure Chemical Company (M = 390 000, $\bar{M}_w/\bar{M}_n \leqslant 1.10$, manufacturer's data for lot No. 3b) was used without further purification.

The solvent *trans*-decalin was obtained by converting a commercial mixture of the *cis*- and *trans*-isomers into the *trans*-form by use of aluminium chloride. The product was washed thoroughly with water and dried over sodium metal. The final purification was made by fractional distillation under reduced pressure in an atmosphere of nitrogen. The purity of *trans*-decalin used was determined as 99.5% by gas chromatography.