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STRUCTURAL ANALYSIS OF CYCLOSPORIN-A BY RAMAN TECHNIQUE

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Abstract

The Raman spectrum of cyclosporin-A has been recorded in the solid phase. The conformation of the molecule has been discussed on the basis of Raman data. It has been concluded that in agreement with X-ray data, cyclosporin-A molecule has an antiparallel β- pleated sheet conformation. It may have some reverse β- turn structure in what has been assigned to a random coil conformation.

Introduction

Cyclosporin-A is a cyclic undecapeptide molecule with the molecular formula $C_{62}H_{111}N_{11}O_{12}$ [1]. It is a metabolite which is extracted from the species of fungi Triohoderma Polysporum (Link ex pers) Rifai. It was found that this molecule has powerful immunosuppressive properties [2], and has provided good results in organ transplantation [3].

As far as the chemistry of cyclosporin-A is concerned, it consists of eleven amino acid residues all of which, except one, have the L configuration. One of the amino acid residues, the D-alanin, has D configuration [4, 5]. One amino acid which is located in position 1 and composed of nine carbon atoms with a C=C band has been isolated for the first time in cyclosporin-A molecule [4].

Although some NMR [1] and X-ray [5] studies have been reported, no vibrational analysis and in particular Raman data is available for this molecule yet.

In our study we have investigated the conformation of this molecule using Raman spectroscopy technique.

Experimental Section

A sample of cyclosporin-A was kindly gifted by the St. James Hospital Leeds and was used without further purification. Dihydro-cyclosporine was prepared using the hydrogenation process with Paladeium as a catalyst.

All Raman spectra reported here have been recorded from solid phase using either a SPEX 1401 double monochromator or Jobin-Yvon Raman microprobe. The instruments were equipped with EMI (SREX) and RCA Type 031034 (Jobin-Yvon Raman microprobe) photon detection systems. They incorporate the PET Type 1502 computers. Excitation frequency at 488.0 nm was obtained from 171 Ar* laser (SPEX) and 164 Ar* laser (Raman microprobe). The position of Raman bands of cyclosporin-A were calibrated against the emmision radiation of a neon lamp.

Keywords: Cyclosporin-A, Amid-Ivibration, Random coil

Results and Discussion

Fig. 1 shows Raman spectrum of cyclosporin-A in the wavenumber shift range 1000-1800 cm^{-1}. Fig.2 shows Raman spectrum of dihydrocyclosporin-A in the wavenumber shift range 1000-1800 cm^{-1}. Figs. 3 and 4 show Raman spectra of cyclosporin-A and dihydro-cyclosporin-A in the wavenumber shift range 1550-1750 cm^{-1} respectively. Fig. 5 shows Raman spectrum of cyclosporin-A in the wavenumber shift range 1190-1300 cm^{-1}.

In the Raman spectra of cyclosporin-A two regions, namely 1650-1680 and 1220-1300 cm^{-1} which are

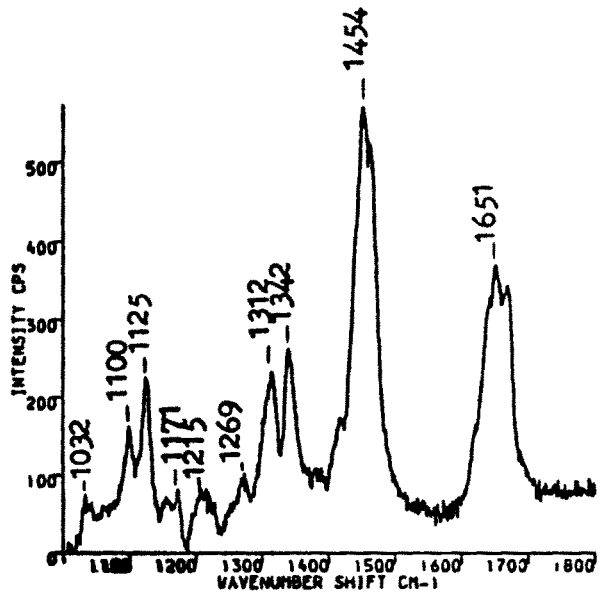

Fig- 1) Raman spectrum of cyclosporin-A in the wavenumber shift range 1000-1800 cm^{-1}.

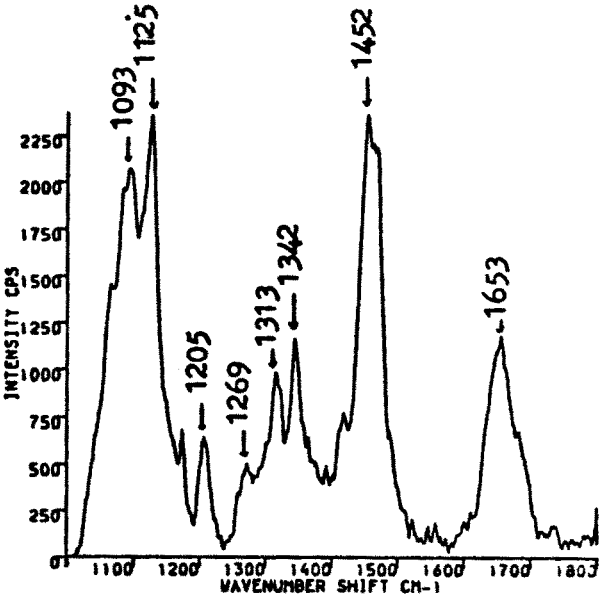

Fig- 2) Raman spectrum of dihydrocyclosporin-A in the wavenumber shift range 1000-1800 cm^{-1}.

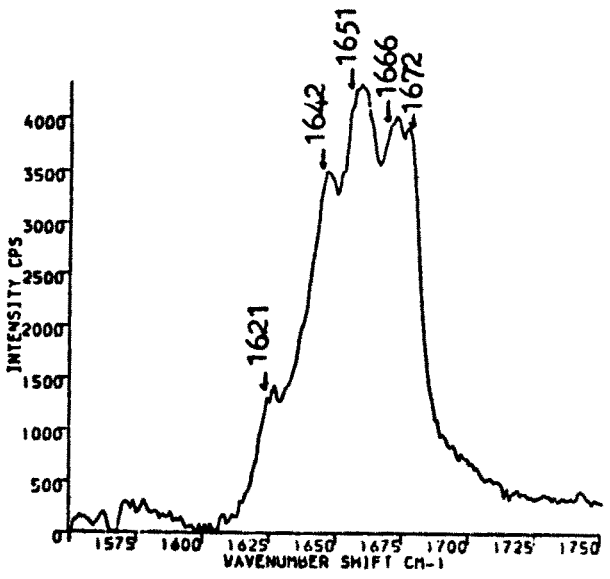

Fig- 3) Raman spectrum of cyclosporin-A in the wavenumber shift range 1550-1750 cm^{-1}.

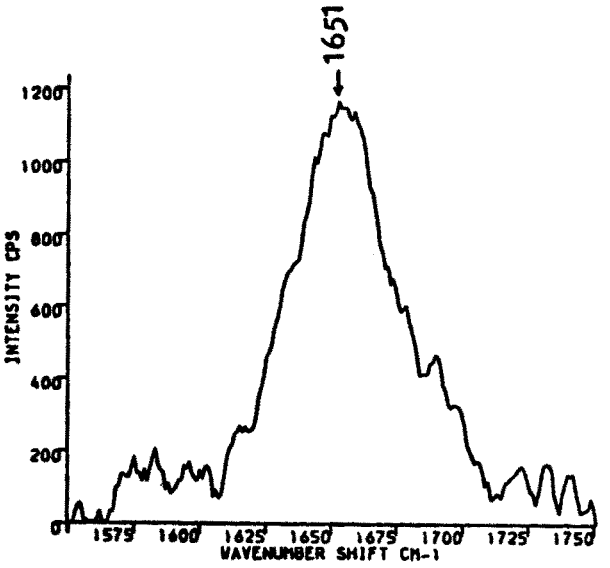

Fig- 4) Raman spectrum of dihydrocyclosporin, in the wavenumber shift range 1550-1750 cm^{-1}.

assigned to amid I and amid III vibrations respectively, are of particular interest to us.

It can be seen from Fig. 1 that there is a strong band at amid -I region and two very weak bands at amid-III region. Under high resolution the amid-I region can be resolved into five components centred at 1621, 1642,

1651, 1666 and 1672 cm^{-1} (see Fig. 3). Cyclosporin-A molecule has a C=C bond (C_9 amino acid), and one of the five bands mentioned above belongs to C=C stretching vibration. An attempt was made to assign the C=C band on the basis of dihydrocyclosporin spectrum, but as it can be seen from Fig. 4 the 1550-1750cm^{-1} spectrum of dihydrocyclosporin is too complicated to make a definite assignment regarding the C=C vibration. According to NMR study, the C=C band in C_9 amino acid is in the trans form [1]. In our Raman spectrum of dihydrocyclosporin, two bands at 1666 and 1672cm^{-1} have disappeared in comparison with the Raman spectrum of cyclosporin in the same region (see Figs 3,4). Based upon the trans nature of C=C band and the observed frequency of this vibration [6] we may tentatively assign the 1672 cm^{-1} to the C=C vibration. (The disappearance of the 1666 cm^{-1} remains ambiguous).

According to X-ray data, cyclosporin-A molecule is composed of two different parts. An antiparallel β-pleated sheet conformation between residues 1-6 and a random coil conformation between residues 7-11 [5]. Koenig has reported a band at 1665 cm^{-1} in amid-I region is a characteristic frequency for oligo-peptides with antiparallel β- sheet structure [7]. We may therefore assign the 1666 cm^{-1} band in the Raman spectrum of cyclosporin-A to the antiparallel β- sheet structure. The amid-III vibration of polypeptides with antiparallel β-sheet structure is reported to be about 1232 [8] and 1247 cm^{-1} [9], but some other frequencies as 1241, 1245, and 1258 cm^{-1} have been assigned to amid -III vibration in solid polypeptides [10]. Mendelsohn has reported that amid-III vibration of polypeptides may be above 1250 cm^{-1} [11]. It can be seen from Fig.5 that in the amid-III region there is a weak band at 1248 cm^{-1}. This observation is consistent with the anti-parallel β- sheet structure of cyclosporin-A molecule.

X-ray data shows that cyclosporin-A molecule has also a random coil structure [5]. This means that we should expect a band at about 1660-1670 cm^{-1}, and at about 1243-1253 cm^{-1} for amid-I and amid-III respectively. In our Raman spectrum we have observed a strong band at 1651 cm^{-1} and a rather weak band at 1269 cm^{-1} (see Figs. 3 and 5). This observation is not consistent with the general random coil structures. In

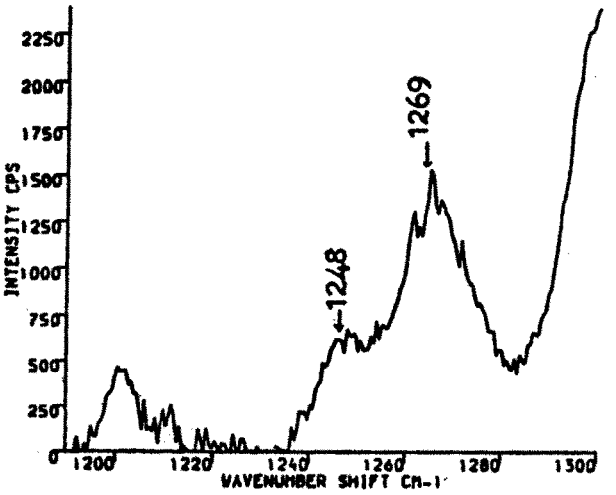

Fig- 5) Raman spectrum of cyclosporin-A in the wavenumber shift range 1190-1300 cm^{-1}.

the conformational analysis of polypeptides the α γ-helical and β- sheet are two well established structures, and any other structures which do not belong to these two structures are considered to be random coil. It has been shown, however, that the β- turns and γ- turns are important structures in polypeptides [12, 13]. It has been shown that small polypeptides containing reverse β-term structure show characteristic amid-I bands near 1649, 1664, 1668, and 1688 cm^{-1}. The amid-III bands appear at a region from 1238 to 1294 cm^{-1} [12, 14]. We may therefore assign the 1651 cm^{-1} and 1269cm^{-1} bands to the amid-I and amid-III reverse β- turn structure in cyclosporin-A molecule respectively. Our study is in good agreement with the X-ray analysis of the molecular structure of cyclosporin-A molecule [5].

We also propose that further studies and in particular a deuterium exchange study would be helpful in the understanding of cyclosporin-A structure.

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