Structural Analysis of Hirudin Using FT-IR and FT-Raman Spectroscopic Techniques

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ABSTRACT: *FT-IR and FT-Raman spectra of hirudin have been recorded from the native solid hirudin. The conformation of the molecule has been discussed on the basis of IR and Raman data. It has been concluded that hirudin molecule has a mixed α-helix and random coil conformation.*

KEY WORDS: *FT-IR, FT-Raman, Hirudin*

INTRODUCTION

Hirudin is a polypeptide with strong anticoagulant activity which is isolated from the salivary glands of the medical leech, *Hirudo Medicinalis.* Hirudin is a potent and highly specific inhibitor of thrombin, an agent being responsible for blood coagulation [1].

The anticoagulant activity of leech saliva was first described as early as 1884 by *Haycraft* [2]. Hirudin was first isolated in 1955 by *Markwordt* [3]. Its aminoacid sequence was determined by *Bagdy* and *Dodt* et al.[4,5]. It consists of a polypeptide chain with 65 residues containing three disulfide bridges [4,5]. It forms a compact N-terminal domain containing disulfide bridges and a long C-terminal tail.

Knowledge of the structure of hirudin plays a central role in understanding the mechanism of thrombin inhibition. The conformations of hirudin in solution have been reported by *Clore* et al. using NMR, distance geometry and restrained molecular dynamics [6]. A two-dimensional NMR study of hirudin has been reported by *Harugama* and *Wuthrich* [7].

No IR and Raman spectroscopic studies of hirudin have been reported. We therefore undertook this study in order to investigate the structure of hirudin in native solid form.

EXPERIMENTAL

Hirudin was obtained from Aldrich (Milwaukee, Wisconsin, USA) in the solid form and was used as received. For the FT-IR measurements a miniature diamond anvil cell (High Pressure Optics Inc., Tucson, Arizona USA) was used, whereas, for the FT-Raman measurements a capillary cell was used.

FT-IR spectra were recorded using a Perkin-Elmer system 2000 FT-IR equipped with P.E microscope. FT-Raman spectra were recorded using a Perkin-Elmer FT-Raman model 2000 equipped with an in-

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dium- gallium-arsenite detector. The excitation wavelength of 1064 nm was obtained from a Nd/YAG laser (I.E.Optomech, model 385).

RESULTS AND DISCUSSION

Fig. 1 shows the FT-Raman spectrum of hirudin in the wavenumber shift range of 400-1800 cm^{-1}. Fig. 2 shows the FT-IR spectrum of hirudin in the wavenumber range of 700-4000 cm^{-1}. Table 1, shows the prominent IR and Raman bands of hirudin.

With regard to the secondary structure of polypeptides in the IR and Raman spectra there are two regions which are of special interest. These are the amide I and amide III vibrations. The amide I vibrations located at 1645-1655, 1660-1665 and 1670-1680 cm^{-1} for α-helix, random coil and β-sheet conformation, respectively. The corresponding amide III vibrations of these conformations are located at 1275(weak), 1245 (broad) and 1235 cm^{-1} (sharp and intense), respectively.

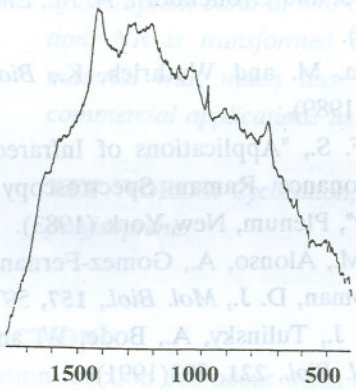

Fig. 1: FT-Raman spectrum of hirudin in the wavenumber shift range 400-1800 cm^{-1}.

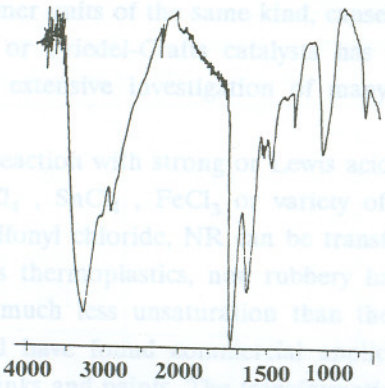

Fig. 2: FT-IR spectrum of hirudin in the wavenumber range 700-400 cm^{-1}.

Table 1: Prominent bands in the FT-IR and FT-Raman spectra of hirudin

IR (cm^{-1})	Raman (cm^{-1})	Intensity
—	763	w
800	—	w
—	1003	m
1067	—	s
—	1126	w
—	1245	w
1280	—	w
—	1326	w
1415	—	w
—	1458	s
1546	—	s
1654	—	vs
2974	—	w(sh)
3135	—	vs

w: weak, m: medium, s: strong, vs: very strong, sh: shoulder

In our FT-Raman spectrum of hirudin (Fig. 1) the 1600-1700 region is completely masked by the intense fluorescence background and is therefore of no use. In the amide III region, however there is a weak and broad band at 1245 cm^{-1} which is an indication of a random coil conformation [8].

In the IR spectra of polypeptides two amide vibrations are active. These vibrations are amide I which are located at the same wavenumbers as in the Raman spectra mentioned previously. The amide II vibration is located at about 1550 cm^{-1} and is normally used in combination with amide I vibrations to elucidate the secondary structure of polypeptides [8].

In our FT-IR spectrum of hirudin (Fig. 2), there is a strong absorption band at 1654 cm^{-1}, which indicates the presence of α-helix conformation. It is also close to the position of random coil conformation. However, the position of the amide II vibration at 1546 cm^{-1} is indicative of an α-helix conformation only [9].

The NMR studies of hirudin [6,7] show that hirudin has a compact N-terminal domain and a disordered C-terminal. Its N-terminal domain is characterized by well-defined turns and two antiparallel β-sheet conformations. In a recent study of the hirudin struc-

ture using a restrained least-squares method, *Rydel* et al. [10] reported three major conformations in the molecular structure of hirudin. They include α-helix, β-sheet and reverse turn conformations.

The α-helix conformation is in good agreement with our results. As far as the reverse turn conformations are concerned, the corresponding amide I and amide III vibrations for this conformation are closely similar to those of the random coil structure (except the α-helix and β-sheet which are well established conformations, any other conformation is considered to be random coil). The weak Raman band at 1245 cm^{-1} in our Raman spectrum of hirudin may therefore be assigned to this conformation. For the β-sheet conformation, our FT-IR spectrum of hirudin, with no absorption band at 1670-1680 cm^{-1}, strongly suggests that such a conformation does not exist in hirudin. It should, however, be pointed out that the previous structural analyses of hirudin have been done in solution, whereas in our study we have used native solid hirudin. This may explain the reason for the absence of β-sheet conformation as is found in hirudin solutions [6,7,10].

CONCLUSIONS

Based upon our FT-IR and FT-Raman spectra of hirudin, we may conclude that the main conformations in the solid state structure of hirudin are α-helix and random coil (or reverse turn). They do not indicate the presence of a β-sheet conformation in native solid form as it has been reported before in solution.

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