

## A Low-Frequency Raman Study of Poly-L-Lysine

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The present communication presents low-frequency Raman spectra from 10 to 300  $\text{cm}^{-1}$  of poly-L-lysine in the solid state and in aqueous solution. Short polylysines (with a degree of polymerization ranging from 15 to 50) are good models for histone tails, which are involved in DNA packaging processes and which act on the nucleic acid through coulombic and hydrogen-bonding mechanisms. The aim of this paper is to discuss a low-frequency experimental Raman investigation of hydrogen bonding in aqueous solutions of poly-L-lysine. The results are compared with spectra of poly-L-lysine in the solid state.

Raman spectra were obtained on a DILOR Z24 spectrometer. 90 or 180° scattering was used. The experimental set-up has been described elsewhere.<sup>1</sup> The spectra in Figs. 1(a) and (b) are given in the so-called  $R(\tilde{\nu})$ -representation,<sup>2–6</sup> eqn. (1), where  $\tilde{\nu}_L$  corresponds to the laser fre-

$$R(\tilde{\nu}) \propto (\tilde{\nu}_L - \tilde{\nu})^{-4} \tilde{\nu} [1 - \exp(-h\tilde{\nu}c/kT)] I(\tilde{\nu}) \quad (1)$$

quency in  $\text{cm}^{-1}$ ,  $\tilde{\nu}$  is the Raman shift,  $I(\tilde{\nu})$  is the intensity at a Raman shift of  $\tilde{\nu} \text{ cm}^{-1}$  and the other symbols have their usual meaning.

Low-frequency vibrations should give direct information upon hydrogen bonding. The forces involved in hydrogen bonding are weak as compared to covalent bonds, thus resulting in rather low vibrational frequencies (i.e. below 300  $\text{cm}^{-1}$ ). Usually the effect of hydrogen bonding is studied by frequency shifts of modes involving ordinary covalent bonds (O–H, N–H, C=O etc.). However, the effect of hydrogen bonding is only a rather small perturbation of the stiff covalent bond. The information obtained from the low-frequency region is more direct, because the corresponding modes involve the hydrogen bond itself. Unfortunately, the low-frequency region is very difficult to investigate experimentally for hydrogen-bonded systems in the liquid state. The far-infrared spectra of aqueous solutions are in general difficult to obtain, and the broad Rayleigh line obscures the band in the low-frequency Ra-

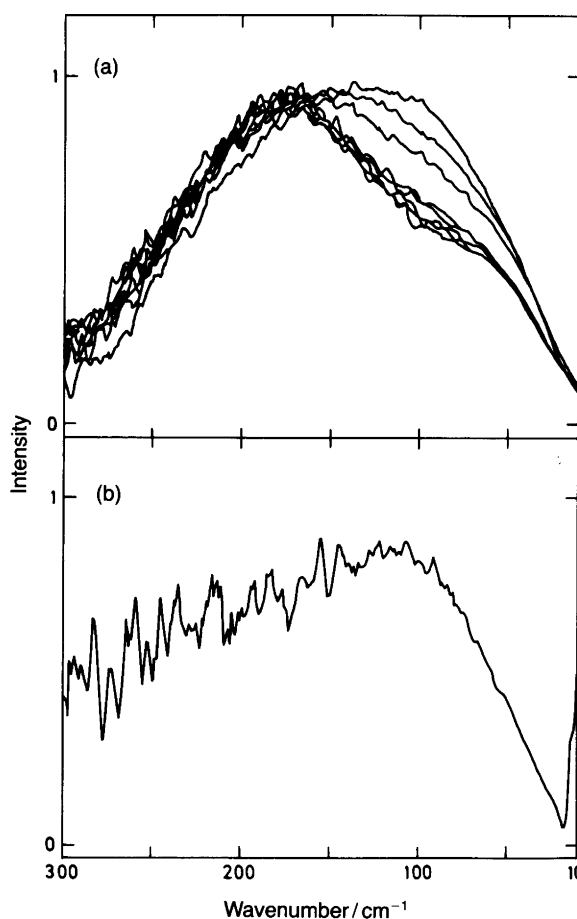


Fig. 1. (a)  $R(\tilde{\nu})$ -Spectra of poly-L-lysine solutions in the buffer tris/HCl. The pH of the buffer was 7.5 and the ionic strength 10 mM. The buffer was subsequently added to 0.8 mg poly-L-lysine. From top to bottom, spectra were obtained of solutions with the following total volumes of buffer added: 0.4, 0.9, 1.9, 3.9, 7.9, 12.9 and 22.9  $\mu\text{l}$ . The bottom curve is the  $R(\tilde{\nu})$ -spectrum of the neat buffer. All spectra were obtained at room temperature. (b)  $R(\tilde{\nu})$ -Spectrum of solid poly-L-lysine (room temperature). As expected for  $R(\tilde{\nu})$ -spectra an increasing noise level with increasing Raman shift is observed. The spectrum was obtained using 180° scattering.

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man spectrum. However, use of the  $R(\tilde{\nu})$ -representation may solve this problem. We have used this technique to study low-frequency bands arising from hydrogen bonding for a number of smaller molecules: acetic acid,<sup>7</sup> formamide,<sup>8,9</sup> *N*-methylformamide,<sup>8</sup> acetamide<sup>10</sup> and *N*-methylacetamide.<sup>11</sup> Also, some biologically interesting molecules have been investigated: nucleosides,<sup>12</sup> nucleotides,<sup>13,14</sup> nucleic acids<sup>15</sup> and proteins.<sup>16</sup> In most cases it was necessary to perform a background correction prior to calculation of the  $R(\tilde{\nu})$ -spectrum in order to reduce fluorescence from unavoidable impurities. We have proposed use of both the Stokes and anti-Stokes sides of the Raman spectrum.<sup>1</sup> However, a disadvantage of this approach is that the signal-to-noise ratio is less in the anti-Stokes side, making studies of weak Raman scattering more difficult. For this reason we have, in the present work, chosen only to investigate the Stokes side of the spectrum. Consequently we have used the ordinary background correction,<sup>4</sup> in which an intensity corresponding to the value at  $350\text{ cm}^{-1}$  is subtracted prior to calculation of the  $R(\tilde{\nu})$ -spectrum according to eqn. (1) [Fig. 1(a)].

The spectra in Fig. 1(a) were obtained of poly-L-lysine solutions in a tris/HCl buffer. The pH of the buffer was 7.5 and the ionic strength 10 mM. An amount of 0.8 mg poly-L-lysine was used. The solutions were obtained by a subsequent addition of the buffer until the following total buffer volumes were reached: 0.4, 0.9, 1.9, 3.9, 7.9, 12.9 and 22.9  $\mu\text{l}$ . An ordinary capillary tube for m.p. measurements was used as a cell. This technique allows use of only a small amount of sample, and thus limits the cost of expensive biochemicals. The maximum intensity in a given spectrum is normalized to the same value for all spectra; thus no comparison of absolute intensities should be performed between different spectra.

Around  $100\text{ cm}^{-1}$  intensity differences are observed between the curves shown in Fig. 1(a). Evidently the intensity of the band with a maximum at ca.  $100\text{ cm}^{-1}$  is proportional to the lysine concentration. Thus this band must be assigned to a mode involving the polypeptide. At least two possibilities seem to exist. The band may be assigned to a mode involving atoms in the CO–HN hydrogen-bonded system. This is in accordance with our observations for liquid amides<sup>16</sup> and our preliminary conclusions for aqueous solutions of lysozyme.<sup>16</sup> However, for nucleosides and nucleotides a rather broad band was assigned to a mode involving hydrogen bonding of the biomolecule to water.<sup>12,13</sup> In order to investigate which mode is observed in

aqueous polylysine the spectrum of solid poly-L-lysine was recorded.  $180^\circ$  scattering was used and the resulting  $R(\tilde{\nu})$ -spectrum is shown in Fig. 1(b). In fact a band is observed with a maximum around  $100\text{ cm}^{-1}$ , which points towards an assignment to a mode involving intrachain hydrogen bonds. It should be emphasized, however, that a small amount of water in the solid cannot be excluded, because no attempts were performed to prepare a completely water-free sample.

In fact, it is worth stressing that, whatever the origin of the hydrogen bonding (resulting either from association with the surrounding water or from intrachain interaction), bonds involved in polylysine must break in order to allow association with DNA; hence, hydrogen bonding is most significant to the interaction process.

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