INFRARED ABSORPTION SPECTRA OF MONOMERIC AND POLYMERIC FORMS OF BOVINE SERUM ALBUMIN

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Received June 24, 1966

Many workers have attempted with some success to find differences in the infrared spectra of plasma proteins which would be of diagnostic value in the study of diseases (Blout and Mellors, 1949; Agnew et al., 1952; Benedict, 1954; Wegmann et al., 1957; Stewart et al., 1959, 1960, 1961). Other workers (Klotz et al., 1949; Saroff et al., 1953; Cook et al., 1954; Beer et al., 1959) using purified proteins have derived spectra-structure correlations, most of which are within the wavenumber range 5000-1000 cm^{-1}. The spectroscopy of proteins in the infrared region has been reviewed in detail by Bellamy (1958), Fraser (1960), and Rao (1963). The availability of procedures for separating monomeric and polymeric forms of Bovine Serum Albumin (BSA) (Pedersen, 1962) suggested the possibility of differentiating between these molecular species by infrared methods.

Although a variety of specimen preparation techniques have been used by the above workers to obtain the infrared spectra, the spectra are comparable from one paper to another. A technique used frequently for preparing specimens for analysis in our laboratory is a micro version of the potassium bromide (KBr) disc method (McNiven, 1965; Caspi et al., in press). A macro version of the

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KBr method has been applied to proteins by Cook et al. (1954). Since the proteins were available as lyophilized solids, we prepared our specimens by the micro KBr method. In this report we shall show that polymeric forms of BSA can be distinguished from the monomeric species by infrared absorption procedures.

Bovine Serum Albumin obtained from Armour Pharmaceutical Company was separated into fractions (Pedersen, 1962) on a 90 cm G-200 Sephadex column. Evaluated in the analytical ultracentrifuge, one fraction consisted chiefly of BSA in monomeric form whereas other fractions consisted of mixtures of monomer, dimer and trimer.

The lyophilized protein was added to a few milligrams of previously ground and dried KBr in a boron carbide micro mortar, and the mixture was ground gently by hand for about a minute. A small rectangle of blotting paper with a central hole was placed on a hardened steel block. The hole was filled to just above surface level with the sample mixture and covered with another hardened steel block. The assembly was held in a hydraulic press at 8,000 lbs. pressure for 30-60 seconds after which the pressure was released and the upper block removed (McNiven, 1965; Caspi et al., in press). The transparent pellet in its blotting paper holder was inserted in a Beckman IR-7 grating spectrometer equipped with a beam condenser. An attenuating comb was used in the reference beam to compensate for energy losses due to light scattering and small sample size.

The spectrum of BSA is dominated as expected by the absorption of the peptide linkages of the "backbone." Correlations to date for the infrared spectra of proteins are given by Saroff et al. (1953), Beer et al. (1959), and Rao (1963). Small differences between protein spectra might be expected due to the frequency and distribution of individual amino acid residues and to the related secondary and tertiary structural features of the molecule. Such differences are evident in the weak bands found in the 1200-600 cm^{-1} range, although relatively little attention appears to have been paid to this region. We have examined this region, therefore, using a weight fraction of protein to

KBr of approximately 0.1. The concentrated KBr pellets contained about 300 micrograms of protein. Although the comparatively strong bands in the 1700-1500 cm^{-1} region are lost, being off scale, the weak bands become sufficiently pronounced to be seen clearly.

The monomeric and polymeric forms of BSA examined in this manner are shown in Fig. 1. Small but noticeable differences can be seen between the monomer and the polymer, namely i) There is a definite band at 845 cm^{-1} in the polymer, whereas in the monomer the corresponding absorption appears as a shoulder at 830 cm^{-1}. ii) There is slight but definite increased absorption at 1085 cm^{-1} relative to 1110 cm^{-1} in the polymer. iii) There is a marked increase in absorption at ~ 1100 cm^{-1} relative to ~ 700 cm^{-1} in the polymer. This appears

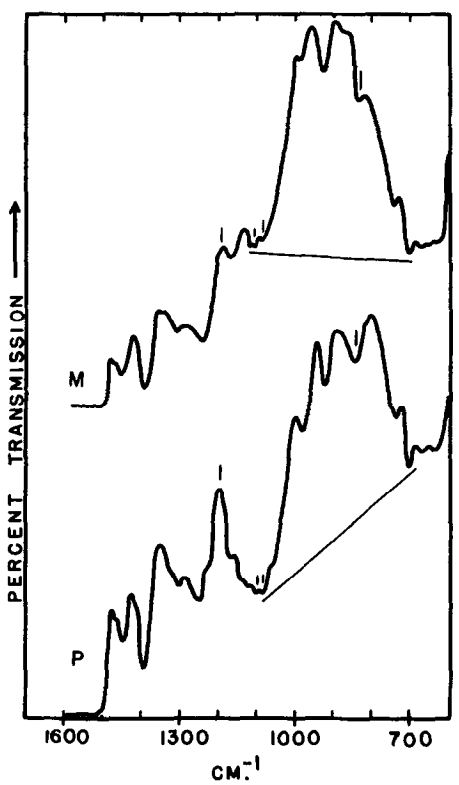

Figure 1. Infrared spectra of eluant fractions from the Sephadex column, (potassium bromide discs).

M) BSA monomer
P) BSA polymer

to be compounded of both an increase at ~ 1100 cm^{-1} and a decrease at ~ 700 cm^{-1}, although the spectra are not quantitative. iv) This change in the 1100 cm^{-1} region is also observed in the 1200 - 1100 cm^{-1} region by the apparent trough at 1195 cm^{-1} in the polymer. Spectra of monomer-polymer mixtures are intermediate between the two in the above specified regions, as expected. The spectra were found to be reproducible.

It is known that changes may sometimes occur in the spectra of compounds when the KBr pellet technique is used, and it was felt by the authors that a check on the reproducibility of our spectra should be made. To do this, and to evaluate the use of the above observations for identification purposes, the following procedure was adopted. Samples of BSA monomer, polymer and monomer-polymer mixture were prepared and checked by ultracentrifugation, the identities of these samples being unknown to the one of us who was to carry out the I. R analyses. These "unknown" samples were then analyzed by the micro KBr technique, identification being attempted on the basis of the above noted monomer-polymer spectral differences, and the results were compared with the true identities of the samples. This procedure was carried out several times, and in every case the samples were correctly identified from their infrared spectra, showing that reproducible spectra were obtained by the above technique. It appears therefore that the infrared procedure we have employed is capable of distinguishing between monomeric and polymeric species of BSA.

Our preliminary observations provoke at least two additional questions. 1) If the individual species were available for examination, could dimer, trimer and tetramer of BSA be distinguished from one another by spectral differences? 2) Do the infrared spectra of polymeric forms of proteins other than BSA differ from those of their corresponding monomers?

Acknowledgments

This investigation was supported by Public Health Service Research Grants FR-05528, HE-02325, and CA-04102.

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