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Methionine sulfoxide formation in proteins: NMR study

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Summary. ^{1}H-NMR spectra of bovine pancreatic trypsin inhibitor (BPTI) both native and oxidized by chloramine T, are reported. The spectrum of the oxidized form is characterized by the appearance of two singlets for methyl group shifted 0.60 and 0.46 ppm downfield with respect to the native form.

Key words: Methionine sulfoxide - NMR - Bovine pancreatic trypsin inhibitor

It is well known that most mature proteins acquire their functioning capability only after post-translational chemical modification. Among the hundreds of reactions that a protein can undergo after the synthesis of polypeptide chain(s), the oxidation of methionine residues has received the attention of biochemists.

Methionine is an amino acid strongly sensitive to oxidation even under mild conditions. When present as methionyl residue on the surface of the protein molecule, it can be selectively oxidized, and its conversion to sulfoxide derivative [Met(O)] has been used to study the influence of this amino acid on the physico-chemical properties of a protein. Moreover, the reaction can easily occur in vivo, either as a consequence of aging or at the site of inflammation where large quantities of oxidants are released by leukocytes; in addition, it is often concurrent with change of biological activity in many proteins (Swaim and Pizzo 1988).

Recent development of sensitivity and resolution of NMR instrumentation allows direct observation of the reaction leading to the sulfoxide formation. We performed ^{1}H-NMR spectra (300 MHz, D_2O, 50° C) of both methionine itself and the methionine residue inside the polypeptide chain. The choice of a model protein fell on bovine pancreatic trypsin inhibitor (BPTI) for its well-known primary structure (58 residues, 1 Met at position 52) and tertiary structure (X-ray cristallography), as well as for the fact that it has been thoroughly investigated by several NMR techniques. Both substrates, which display a sharp, well detectable singlet at 2.42 ppm, were oxidized by chloramine T at neutral pH and, after purification from the by-products, again submitted to ^{1}H-NMR analysis. The disappearance of the 2.42 ppm signal of the singlet for the methyl group of methionine was complete after addition of equivalent amounts of the oxidant, confirming the 1:1 stoichiometry of the reaction (Concetti et al. 1989) with respet to methionine contents. The sulfoxide group formation implies the introduction in the molecule of a new stereogenic center. Methionine sulfoxide displays two very close singlets for the methyl group shifted by 0.60

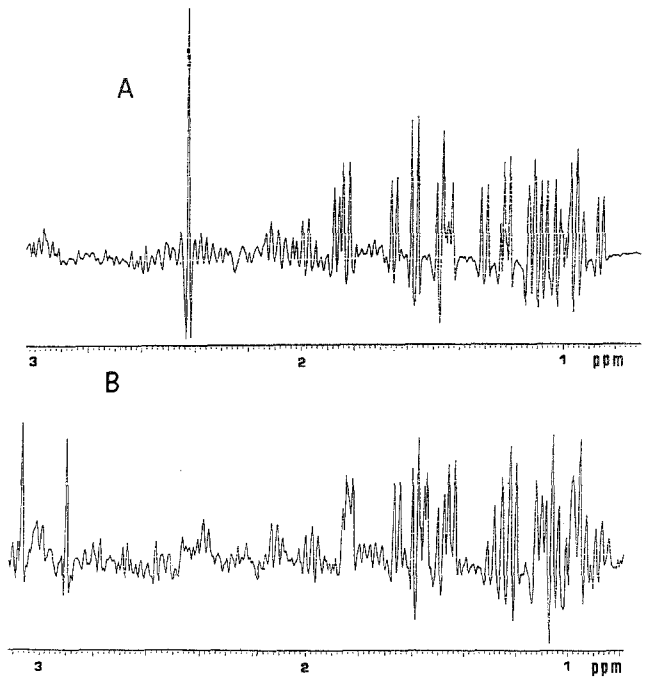

Fig. 1. Spectral region between 0.8-3.0 ppm in the ^{1}H-NMR spectrum at 250 MHz, 50° C of 10 μM solution of both native BPTI (**A**) and oxidized BPTI (**B**) in H_2O, pH = 7.0

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ppm downfield compared to the unreacted methionine (data not shown). Oxidized BPTI shows two singlets shifted by 0.66 and 0.46 ppm with respect to the native protein (Fig. 1). This more relevant chemical shift in the protein is accounted for by the higher chirality of the molecule. Further differences can be observed in the high-field region of the spectrum where the methyl groups resonate. On the basis of previous assignments (de Marco et al. 1977), some methyl groups (e.g. the line at 1.87 ppm corresponding to Thr54, Fig. 1), belonging to residues spatially close to the site of oxidation, undergo either slight chemical shift variation or splitting into two signals; this can obviously be ascribed to new magnetic anisotropy and chirality connected with the sulfoxide group. Some chemical shift differences are also remarkable in methyl groups which are far away from the site of oxidation (e.g. the line at 0.95 ppm corresponding to Ile19, Fig. 1); this can be the consequence of conformational changes produced by the entrance of the highly hydrophylic oxygen atom into the molecule.

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