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Effects of high hydrostatic pressures on secondary structure of acetylcholinesterase with and without carbachol

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Kalogeros G, Wong PTT, Lecelle S, McIver DJ, Philp RB. Effects of high hydrostatic pressures on secondary structure of acetylcholinesterase with and without carbachol. Undersea & Hyperbaric Med 1994; 21(1):1–7.—Ultra-high hydrostatic pressures (to 13 kbar) were applied to acetylcholinesterase (AChE) in the presence and absence of 1 m*M* carbachol (a muscarinic agonist) by means of a piston-and-cylinder system designed for use with Fourier transform infrared spectroscopy. At normal atmospheric pressure, carbachol decreased the number of intramolecular hydrogen bonds and the anti-parallel β-sheet structure. In the absence of carbachol, pressure dramatically increased the number of intermolecular hydrogen bonds but decreased the α-helical, β-sheet, and anti-parallel β-sheet segments. In the presence of carbachol, pressure had the opposite effects, decreasing the number of intermolecular hydrogen bonds and increasing the α-helix: β-sheet ratio. Thus in the absence of an attached ligand, the enzyme molecule was vulnerable to pressure-induced distortions that would most likely impair its function. These effects were observed in the absence of a lipid component, indicating that pure proteins are vulnerable to pressure-induced changes in configuration that could affect function.

pressure, proteins, enzyme, structure, spectoscopy, carbachol, cholinesterase

The technique of pressure-tuning Fourier transform infrared spectroscopy (PT-FTIR) constitutes a unique tool for the study of secondary structural characteristics of complex protein molecules. Pressure distortion reveals otherwise-undetectable information concerning the nature of amide side-chains, inter- and intra-molecular hydrogen bonding, etc., and the coupling of multiple infrared scans to Fourier transform computer analysis constitutes a powerful analytical tool (1). FTIR may also provide relevant information regarding the effects of hydrostatic pressure on biological systems. Although the pressures employed, in the kilobar range, are far beyond those encountered by any living species except the deepest-dwelling ocean creatures, the nature of the spectral patterns generated by increasing hydrostatic pressures constitutes a continuum that may reflect events at lower pressures.

One area of particular interest regarding the biological effects of hydrostatic pressure is its influence on ligand-receptor interactions because this bears on many cell processes, especially neurotransmitter–receptor and substrate–receptor attachments. Carbachol is a muscarinic, cholinergic agonist that is recognized by acetylcholinesterase (AChE) but which is not degraded by it (2). It thus constitutes a useful model of receptor–ligand interaction and the effects of pressure thereon. We report here on the effects of ultra-high hydrostatic pressures on AChE in the presence and absence of carbachol.

MATERIALS AND METHODS

Carbachol and AChE were obtained from Sigma Chemical Co. (St. Louis, MO). For FTIR studies, AChE was dissolved in deuterium oxide (D_2O) to make a 10% solution. To one half of the solution, carbachol was added at a concentration of 1 m*M*. A few microliters of either solution were placed, together with a small amount of α-quartz as a pressure indicator (3), in the 0.37-mm diameter hole of a 0.23-mm-thick gasket mounted on a diamond anvil cell as described previously (1). Infrared spectra were measured at 28°C on a Bomem Michelson model 119 Fourier transform, infrared spectrophotometer with a liquid N_2-cooled mercury telluride cadmium detector. The infrared beam was condensed by a sodium chloride lens onto the pinhole of the diamond anvil cell. Typically, 512 scans (600–4,000/cm) were co-added to produce a spectrum (resolution of 4/cm); therefore all observations were empirical. Pressures were determined from the 695/cm band of α-quartz using previously recorded data (3). In all figures of spectra the Y axis is absorbance. Deconvolution refers to the Fourier transformations designed to augment derivations in the line spectrum that would amplify obvious as well as subtle but important changes in the raw spectra (4).

RESULTS

Figure 1 displays the original (*A*) and deconvolved (*B*) spectra for the amide I band for AChE alone and with carbachol. The conformational structures are dominated by the β-sheet structure (1,636–1,638/cm) with some α-helical segments (1,658/cm). The 1,683-cm band is due mainly to anti-parallel β-sheet substructure. The amide I mode of the intermolecular amide groups also contributes to this band. The 1,611-cm band is due entirely to hydrogen bonding between the amide groups of adjacent protein molecules. Figure 1 shows clearly that carbachol decreases the number of these intermolecular hydrogen bonds as well as decreasing the anti-parallel β-sheet structure.

The effects of pressure (to 13 kbar) on AChE were quite different in the absence (Fig. 2) and presence (Fig. 3) of carbachol. Both figures show the deconvolved amide I bands. In the absence of carbachol (Fig. 2) pressure dramatically increased, in a pressure-dependent manner, the number of intermolecular hydrogen bonds. Conversely, α-helical, β-sheet, and anti-parallel β-sheet segments were all decreased. In the presence of carbachol (Fig. 3), increasing pressure increased the α-helix:β-sheet ratio and decreased the number of intermolecular hydrogen bonds. The anti-parallel β-sheet segments were still decreased.

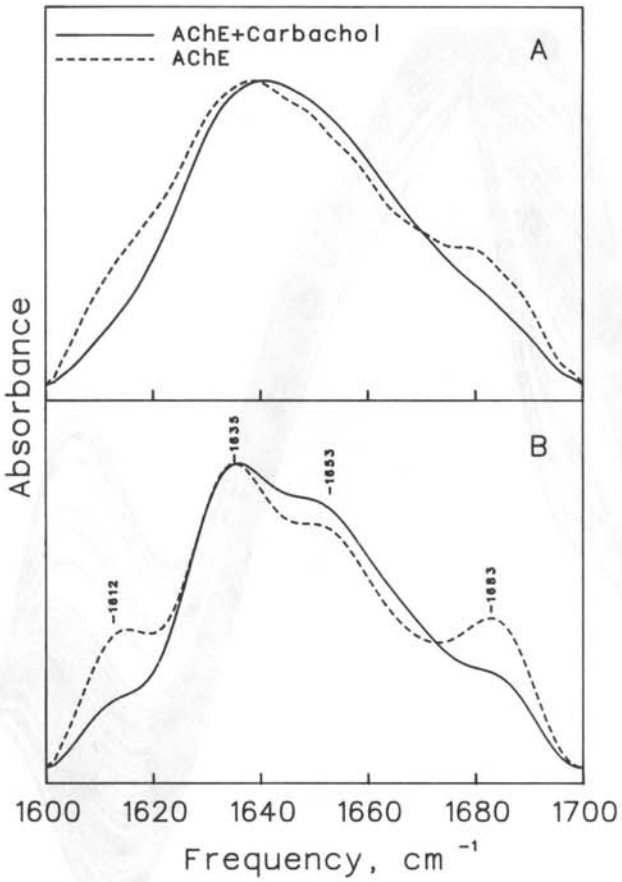

FIG. 1—Infrared spectra of the amide I band of AChE in the presence and absence of carbachol (1 m*M*). *A*, original spectra; *B*, deconvolved spectra with an enhancement of 1.4 and band width of 20 cm.

Figure 4*A*, *B* shows the pressure dependencies [the shifts in frequency peak(s) with increasing pressure] of the amide I component bands for AChE alone (Fig. 4*A*) and with carbachol (Fig. 4*B*). The α-helical contribution to the amide I band for AChE was maintained through the entire pressure range in the presence and absence of carbachol. For AChE alone, the decrease in the intensities of the β-sheet band precluded their accurate measurement above 8 kbar. In the presence of carbachol, the β-sheet structures were stabilized, allowing a slight increase in intensity beyond 8 kbar. Interestingly, hydrogen bonding between AChE molecules was eliminated in the presence of carbachol above 8 kbar as indicated by the peak at 1,610/cm.

DISCUSSION

In the absence of carbachol, the α-helical and β-sheet substructures of Achase were not stable against the application of external pressure, as indicated by the decrease in the amounts of these segments with increasing pressure as well as the decrease in the hydrogen-bond strength of the β-sheet structure with increasing

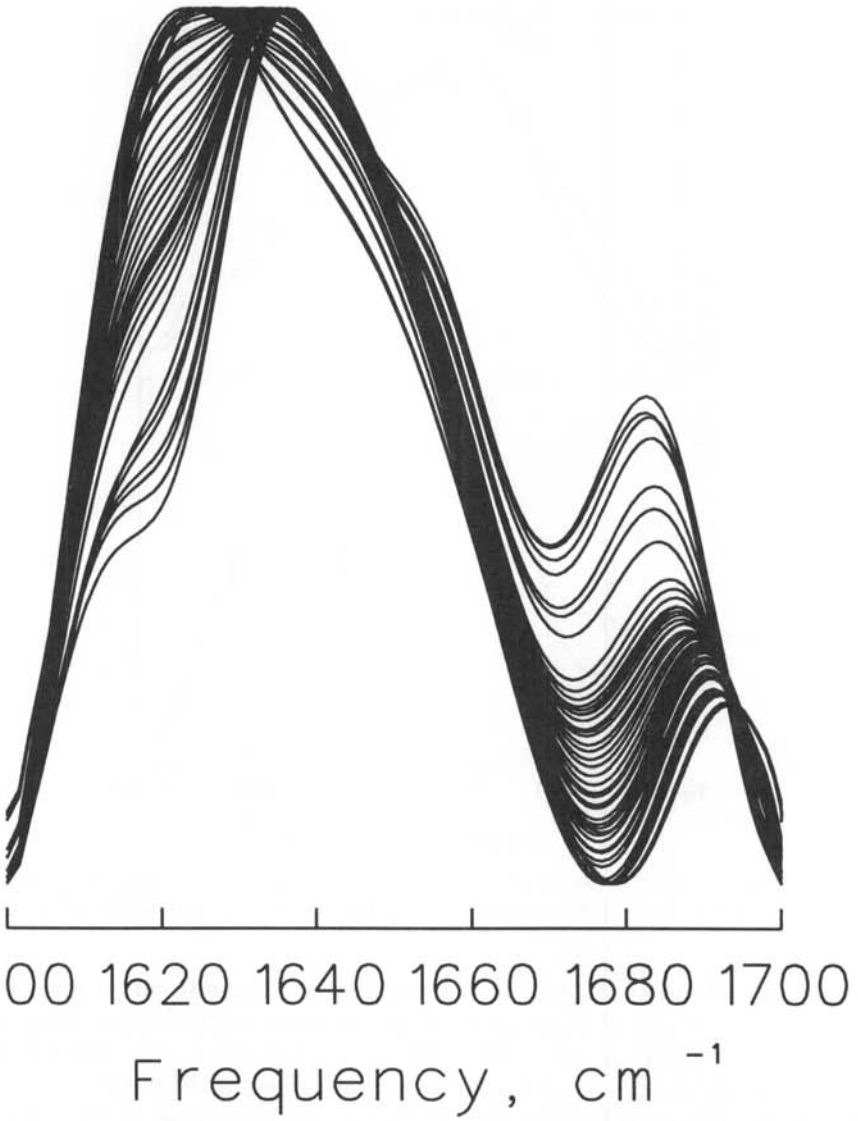

FIG. 2—Stacked contour plots of the infrared spectra for AChE in the presence of carbachol are shown at increasing pressure from normal atmospheric pressure (*bottom*) to 14 kbar (*top*).

pressure. This is evident from the increase in the frequency of the β-sheet band with increasing pressure. Carbachol had the effect of a) protecting the α-helical and β-sheet structures from pressure denaturation, b) protecting the protein molecules from intermolecular aggregation by decreasing the strength of the intermolecular hydrogen bonds of the amide groups, and c) protecting the protein molecules from pressure-enhanced intermolecular aggregation. Put another way, in the absence of an attached ligand, the enzyme molecules were vulnerable to pressure-induced loss of structural integrity and to intermolecular aggregation, both of which would affect function. In

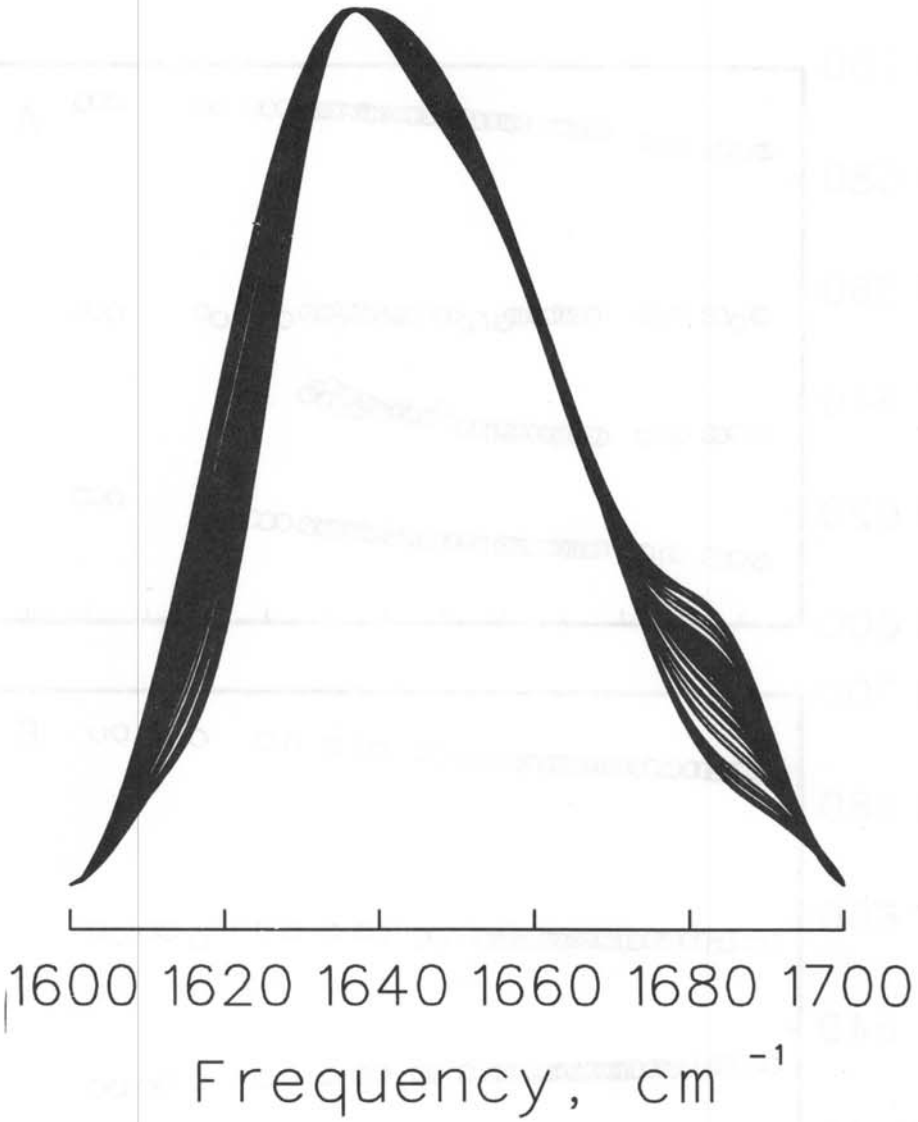

FIG.3—Stacked contour plots of the infrared spectra for AChE in the presence of carbachol are shown at increasing pressure form normal atmospheric pressure (*bottom*) and 13 kbar (*top*).

a previous study using aa lipid–albumin complex as a model membrane, we found that clofibrate, which binds to sites on albumin, reduced the pressure enhancement of the lipid–albumin interactions (5). The present study indicates that the presence of a lipid component is not essential to demonstrate an interaction of pressure with a ligand–receptor complex.

Inhibition of enzyme activity by hydrostatic pressure has been previously demonstrated. Lum and Zimmerman (6) found that 0.34 kbar significantly inhibited the activity of a number of dehydrogenases when measured 5 min after decompression.

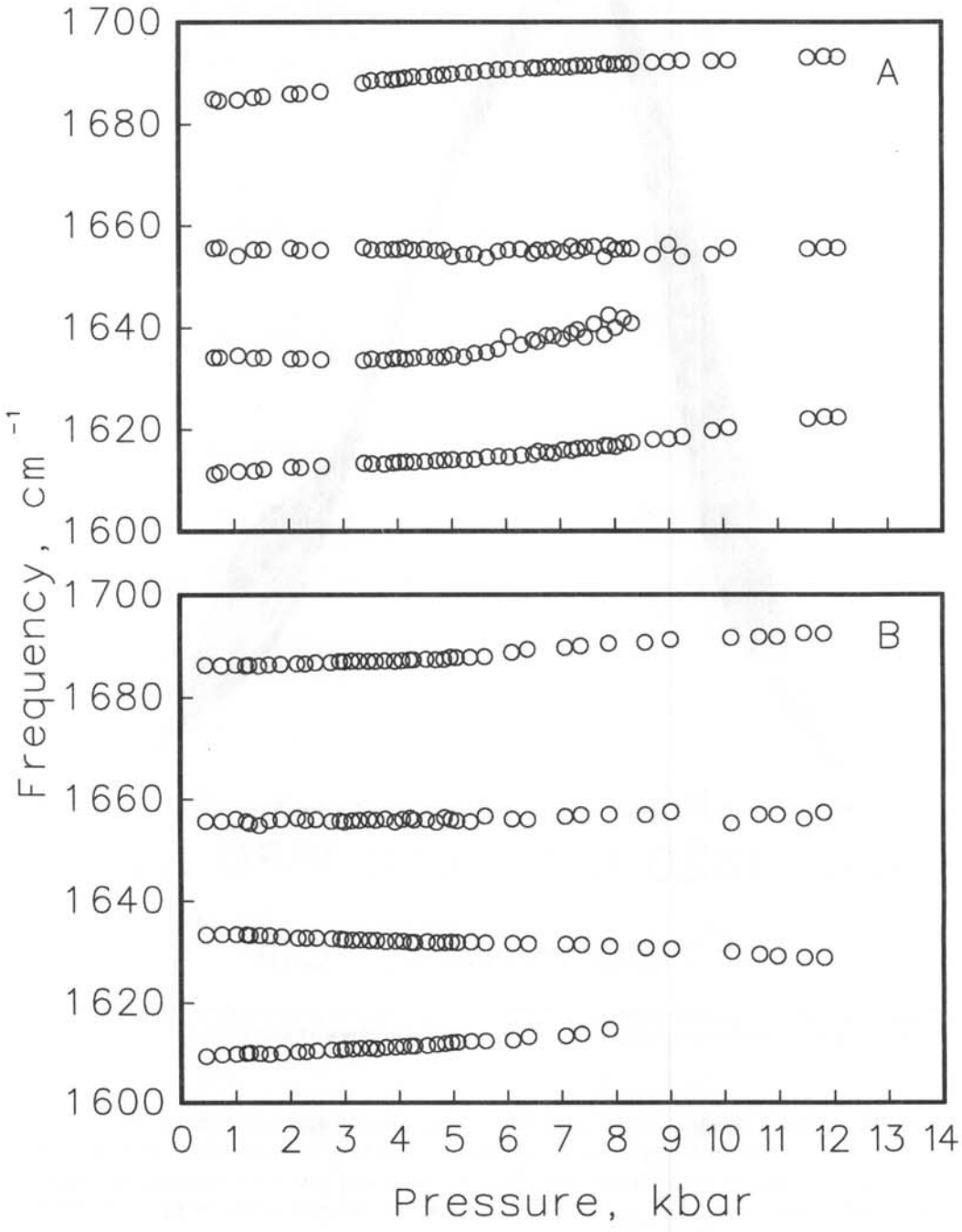

FIG. 4—Pressure dependencies of the frequencies of the amide I component bands are shown for AChE alone (*A*) and in the presence of carbachol (*B*).

Others have shown that He pressure in the 300-bar (0.3 kbar) range inhibited the binding of tritiated acetylcholine (ACh) to electroplaque ACh receptors or ACh-binding protein (7, 8).

Together these findings support the hypothesis that pressure interferes with a variety of physiologic functions by affecting receptor-ligand interactions required for enzyme activity and neurotransmission. The present work suggests that this may occur through an alteration of receptor protein secondary structure.

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