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INTERACTION OF ACETYLCHOLINE AND β-METHYLACETYLCHOLINE WITH ALUMINIUM OXIDE SURFACE STUDIED BY INELASTIC ELECTRON TUNNELING SPECTROMETRY

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SUMMARY :

Inelastic Election Tunneling Spectrometry (IETS) yielded useful information on the interaction of Acetylcholine and its analog β-Methylacetylcholine with an alumina surface. The spectra obtained are discussed in comparison with infrared and Raman spectra of the same molecules in solid state and in aqueous solutions studied previously by the authors. It is shown that under certain conditions Al_2O_3 catalyses the hydrolysis of molecules as does the Acetylcholine-esterase enzyme on the postsynaptic membrane or a basic aqueous medium with a pH greater than 9.

INTRODUCTION :

Among the many factors related to the biological activity of acetylcholine, Ach $[(CH_3)_3\overset{+}{N}CH_2CH_2OCOCH_3]$, as a chemical transmitter in the nervous system, a particularly prominent role is attributed to its conformation. Thus the solid state and solution conformations of Ach and related molecules have been the subject of extensive studies both experimental and theoretical (1, 2, and references therein). In particular, the conformation of the molecule when attached to the postsynaptic membrane has been the object of many hypotheses but no experimental work has been done.

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We used Inelastic Electron Tunneling Spectrometry (IETS) to gain some knowledge in this field. The method is particularly well adapted for adsorption studies and can detect vibrational modes of organic molecules that are active in both infrared (IR) and Raman (R) spectrometry (3). The details of the vibrational spectra can be interpreted in terms of molecular conformation and of perturbations due to adsorption. Other advantages of the method are that it has a resolution better than 0.5 meV (4 cm^{-1}) and a very high sensitivity (down to 1/30 monolayer) (4).

EXPERIMENTAL :

Acetylcholine and its more rigid analog β-Methylacetylcholine β-MeAch[$(CH_3)_3N^+CH_2(CH_3)CHOCOCH_3$] were obtained from Sigma Chemical Company as AchI and MeAchBr compounds and were used without further purification. The molecules are deposited on the oxide layer of an Aluminium-Oxide-Lead electron tunneling junction. Under an applied voltage, electrons tunnel from one metal to the other and can excite a vibrational mode of the organic compound producing an abrupt change in the dynamic conductance $\sigma = dI/dV$ and a peak in the second derivative $(1/\sigma) \cdot (d\sigma/dV)$ at a voltage corresponding to the energy of the vibration.

Ach and β-MeAch were deposited in aqueous solutions. The aluminium electrode was very slightly oxidized in an O_2 plasma, then removed from the vacuum system for doping which was done under two different conditions. In the first case (I), 10^{-4} molar aqueous solution of AchI or β-MeAchBr was deposited onto the Al_2O_3 surface for two minutes and then rinced with distilled water (Fig. 1). In the second (II), the molecules were deposited as indicated above but without being rinced (Fig. 2) : in both cases excess liquid was blown off with a jet of Argon.

RESULTS AND DISCUSSION :

As seen in Fig. 1 and 2, the two series of spectra are quite different whereas in each series the spectra of Ach and β-MeAch are very similar. Under the first conditions the spectra (Fig. 1) present two large peaks at 178 meV (1435 cm^{-1}) and 198 meV (1596 cm^{-1}) that belong neither to the IR nor the R spectra of Ach and β-MeAch (1,2), while the peaks characteristic of the νC=O vibration at 215 meV (1735 cm^{-1}) and of the ν_aC-O vibration at 155 meV (1245 cm^{-1}) are absent. This is comparable to what we observed by Raman spectrometry after the non-enzymatic hydrolysis of Ach in a basic aqueous medium (5) : the final products of the reaction were acetate ions and choline. We also determined the IETS spectrum of acetate ions chemisorbed on Al_2O_3 in a previous experiment (3) : all of its features (Fig. 1,C) are present in the

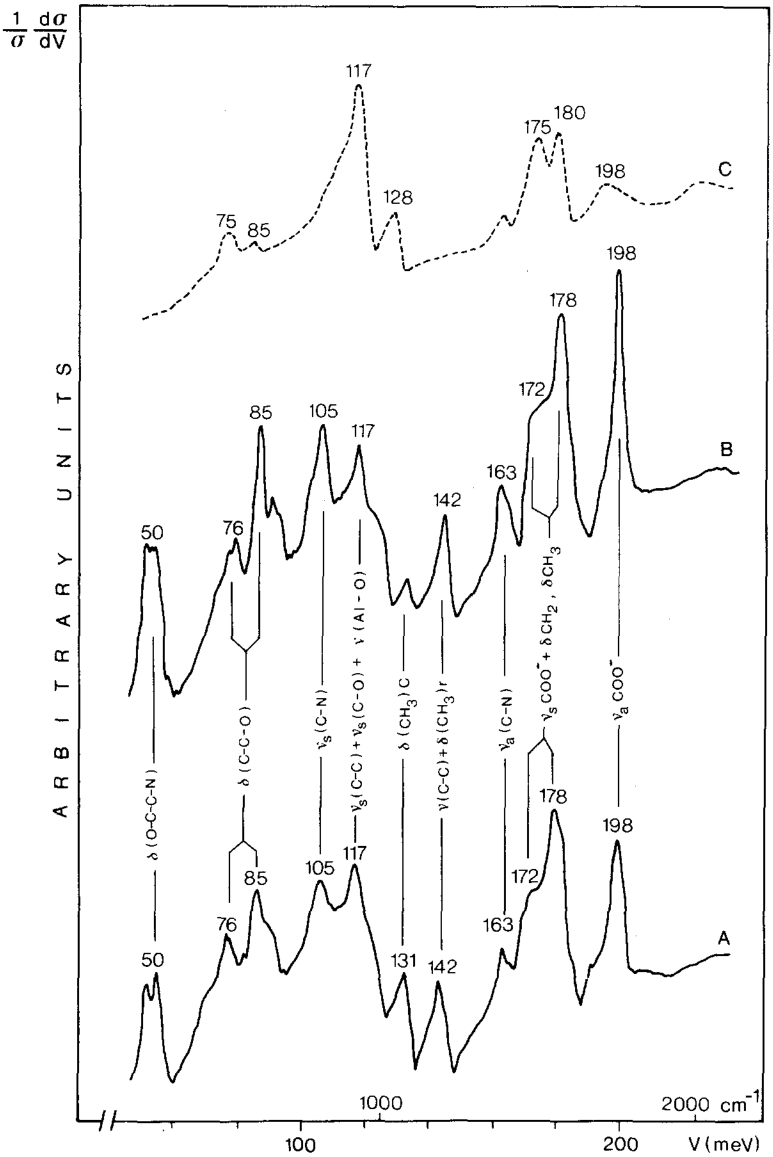

FIG. 1 : IETS spectra of AchI (A) and β-MeAchBr (B) prepared under conditions I (see text). The molecules have been hydrolysed as confirmed by comparison with the IETS spectra of the acetate (C) formed in the junction doped with acetic acid.

Ach and β-MeAch spectra (Fig. 1,A and B). Thus we assign the peak which appears at 198 meV to the $\nu_a COO^-$ vibration of the acetate ion (3,5,6) and the one at 178 meV to a mixture of $\nu_s COO^-$ and δCH_2 and δCH_3 modes. This, taken together with the disappearance of the $\nu C{=}O$ and $\nu_a C{-}O$ modes, leads us to the

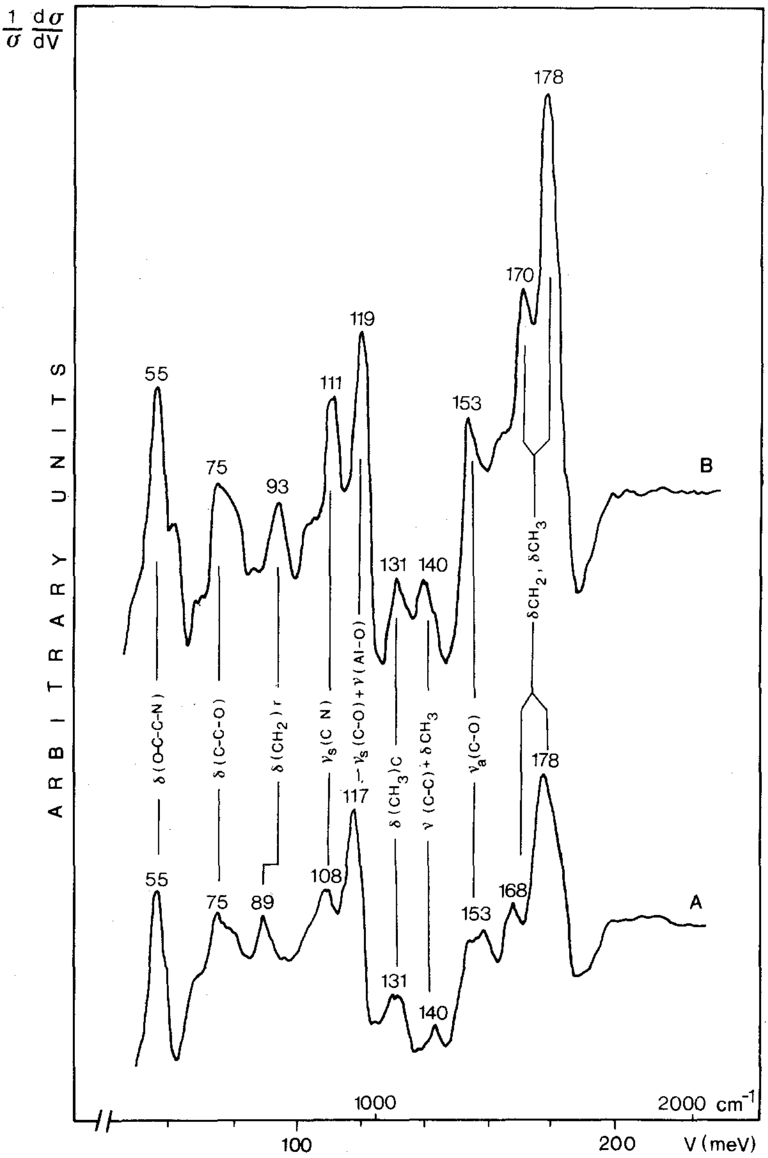

FIG. 2 : IETS spectra of AchI (A) and β-MeAchBr (B) prepared under conditions II (see text). The molecules are not hydrolysed although the spectra are perturbed by adsorption.

conclusion that under our first conditions Al_2O_3 acts as catalyser for the hydrolysis of Ach and β-MeAch, as does the Acetylcholinesterase enzyme on the postsynaptic membrane or as does a basic aqueous medium with pH greater than 9 (5). The interaction of the liberated choline with Al_2O_3 is indicated by deplacement or intensity changes of almost all peaks assigned to the choline vibrations (1,2). Studies are in progress on their interpretation.

In the spectra recorded under our second conditions, shown in the Fig. 2, we do not find the strong peak of $\nu_a COO^-$ at 198 meV (1596 cm^{-1}). Therefore we assign the peak present at 178 meV (1434 cm^{-1}) to the bending CH_2 and CH_3 vibrations only (1). The vibration $\nu_a C$-O, absent when hydrolysis of Ach and β-MeAch has taken place, is now present. It is clear that under the second conditions the adhesion of Ach and β-MeAch onto the Al_2O_3 surface does not lead to their hydrolysis. Nevertheless the adsorption induces a modification of the molecules : the νC=O vibration at 215 meV is not observed and there are perturbations in the skeletal modes of the molecules, which are now being studied.

In conclusion, we believe that this method provides important information on the interactions of Ach and β-MeAch with the alumina surface. Work is underway to interpret this information and in particular to explain the mechanism of hydrolysis as well as the non-hydrolytic interaction. It is our hope that a good understanding at the molecular level of these interactions may be useful in the understanding of the "active conformation" of Ach at the membrane surface.

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