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NMR Evidence for an Acetylcholine ATP Complex

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SUMMARY

The interaction of acetylcholine with ATP was studied using proton NMR. T_1 measurements indicate the formation of a complex with a probable composition of four (4) acetylcholine molecules for each ATP molecule.

INTRODUCTION

It has been known for several years that epinephrine is stored with ATP and there have been several NMR studies of this complex (1,2). Until recently the nature of acetylcholine storage vesicles has remained a mystery. It has now been demonstrated that acetylcholine, in some systems, is stored with a specific protein and ATP (3,4). While a previous study failed to show any interactions between acetylcholine and ATP (5), our work has demonstrated the formation of an acetylcholine ATP complex with a definite stoichiometry. We feel that in the light of the recent evidence that acetylcholine is stored with ATP this complex should be of great interest to workers in the field.

In order to study the interaction of acetylcholine and ATP T_1's were determined for acetylcholine alone, and in different mole ratios with ATP.

Proton relaxation results from magnetic dipole coupling between neighboring spins on the same molecule, and the relaxation rate $(T_1)^{-1}$ is directly proportional to the reorientational

correlation time, τ_θ, for the vector joining the pair of coupled spins (6)

$$(T_1)^{-1}_{intra} = \frac{3\gamma^4 \hbar^2}{2r^6} \tau_\theta. \quad (1)$$

In equation (1) γ is the gyromagnetic ratio of the proton ($26{,}751 \frac{rad}{sec\text{-}gauss}$), and r is the internuclear separation. Complex formation leads to a substantial increase in τ_θ for protons on the acetylcholine, and thus the T_1's of these protons provide a sensitive probe of binding, even in the presence of rapid chemical exchange and when electronic perturbations caused by the binding are vanishingly small. For the methyl protons τ_θ will in general reflect the rates of both internal rotation and overall molecular reorientation. When intramolecular methyl rotation is relatively free, the protons on these groups provide a less sensitive binding probe than do the methylene protons, which are constrained to reorient with an axis system fixed in the complex. For all the protons, the r^{-6} dependence of $(T_1)^{-1}$ upon internuclear separation ensures that only those protons nearest the one monitored contribute significantly to T_1 (increasing the internuclear separation by 50% lessens the $(T_1)^{-1}_{inter}$, contribution by 88%). In addition to the intramolecular dipole contribution described in equation (1), an intermolecular component $(T_1)^{-1}_{inter}$, may also be present due to dipole coupling to protons on ATP and water. The latter interaction has effectively been suppressed in the present experiments by examining solutions lyophilized in D_2O; relaxation due to ^2H-^1H coupling is only 1/64 as efficient as that of proton-proton coupling. Intermolecular coupling to protons on ATP is probably quite small compared to intramolecular coupling, but in any event this effect causes an additional shortening of T_1 upon complexation and thus, if anything, auguments the sensitivity of the technique.

MATERIALS

Acetylcholine chloride, D_2O, and Na_2 ATP were purchased from Sigma. These were prepared by 3 cycles of freeze-drying from D_2O. All samples were brought to pD 7.0 $\pm$ .2 with NaOD. This is equivalent to pH 7.4 $\pm$ .2 (7). All samples were degassed by several cycles of freeze-pump-thaw. The concentration of acetylcholine was .33 molar in all samples except the 0.5:1 which was .167 M in acetylcholine and .33 M in ATP. All work was done at 23° $\pm$ 2°.

METHODS

Partially relaxed spectra were used to measure T_1's. These were generated by a 180°-t-90° pulse sequence (8,9), t increasing from 0.1 second to 5.0 seconds in 13 steps. A delay of 20 seconds was used between each pulse sequence, five free induction delays were averaged for each spectra. A JEOL JNM PFT-100 100 MHZ spectrometer with a Digilab computer system was used to collect the data. T_1's were measured for the methyl attached to the carboxyl carbon (Δ), the methyls attached to the nitrogen (o), and the methylene attached to the nitrogen (●). The resonances of the methylene attached to oxygen were partially obscured by the ATP and could not be used for quantitative measurements.

RESULTS

Figure 1 shows a plot of the change in T_1's as the ratio of acetylcholine to ATP is changed. The addition of ATP causes a shortening of the T_1's indicating binding. The initial change is rapid but saturates when the ATP/Acetylcholine ratio exceeds about a 1 to 4 mole ratio. All of the protons monitored show similar changes upon complex formation. This indicates that internal methyl rotation is not rapid compared to overall mole-

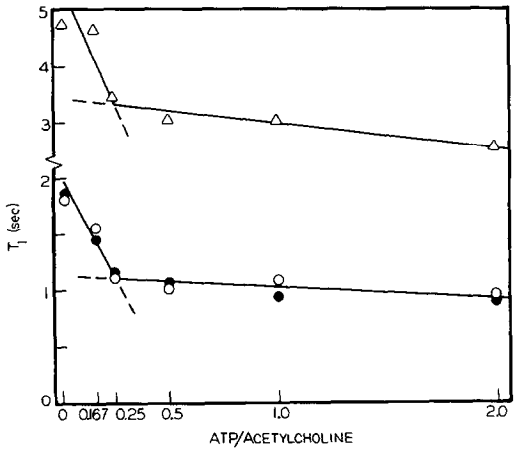

Figure 1. Acetylcholine proton T_1's as a function of ATP/acetylcholine mole ratio for CH_3-C (Δ), CH_3-N (O) and CH_2-N(•).

cular reorientation and that both the methyls and the methylenes are suitable binding probes.

DISCUSSION

Since T_1 is a sensitive measure of complexation, the shorting of T_1 values as ATP is added to acetylcholine is proof of the formation of a complex. As also shown in Figure 1 there is a strong suggestion that this complex is composed of 3, or more likely 4, acetylcholine molecules to each ATP molecule. The finding of this complex is interesting both because of the information it may provide on the storage of acetylcholine, and because it can serve as a model system to investigate the pharmacology of acetylcholine interactions.

1) Weiner, N. and Jardetzky, O. (1964) Arch. Exptl. Pathol. Pharmakol. 248, 308-318.

2) Tuck, L.D. and Baker, J.K. (1973) Chem-Biol. Interactions 7 355-366.

3) Whittaker, V.P., Dowdall, M. J., and Boyne, A.F. (1972) Biochemical Society Symposia 36, 49-68.

4) Silinsky, E. M. and Hubbard, J. I. (1973) Nature 243, 404-405.

5) Behr, J. and Lehn, J. (1972) Biochem. Biophys. Res. Commun. 49, 1573-1579.

6) Abragam, A. (1961) Principles of Nuclear Magnetism p. 300, Oxford U. Press, London.

7) Glasoe, P.K. and Long, F. A. (1969) J. Phys. Chem. 64, 188-191.

8) Vold, R.L. et al., (1968) J. Chem. Phys. 48, 3831-3832.

9) Freeman, R. and Hill, H. W. D. (1969) J. Chem. Phys. 51, 3140-3141.