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Taurine-calcium interaction measured by means of ^{13}C nuclear magnetic resonance

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Despite the fact that many of the physiological roles of taurine are still obscure, a series of experimental data have been published suggesting that taurine can have a function in the modulation of calcium fluxes at cardiac level [1–3] and at the level of liver mitochondria [4].

It was suggested that the interference of taurine with calcium fluxes could be interpreted as the result of the formation of a calcium-taurine complex [1–4]. This hypothesis was rejected in a paper of Igisu *et al.* [5].

We have further investigated this point by means of Fourier Transform ^{13}C nuclear magnetic resonance (^{13}C-FT-NMR). In recent years it was in fact demonstrated that this is a powerful tool for the study of weak ligand–metal interactions. [6].

MATERIAL AND METHODS

Chemicals. Taurine was purchased from Schuckardt. Calcium chloride (Suprapur), deuterium oxide, deuterium chloride, dioxane and tetraethylammonium chloride from Merck. Tetraethylammonium chloride was purified from heavy metal impurities by repeated crystallizations.

Nuclear Magnetic Resonance measurements. The ^{13}C NMR spectra were obtained with a Brucker WH90 Fourier transform NMR spectrometer, operating at 22.63 MHz and at a probe temperature of 37°. Pulse duration was chosen in order to avoid any saturation effect. 5000 signals were accumulated for every experiment and coherently added in a Nicolet BNC12 digital computer. All the spectra were recorded while decoupling with a broad band modulated proton decoupling frequency. Chemical shifts were measured from dioxane as internal reference standard. Deuterium oxide was always used as a solvent and for internal heteronuclear lock operations. Sweep width was 6 KHz. A width of 0.6 KHz was used for relaxation times measurements. Relaxation times were obtained graphically by line width measurements, using solution free of oxygen, obtained with a nitrogen stream.

The precision of the instrument was such that repeated measures of the chemical shifts of the same sample differ less than 0.022 ppm. The pH of the solutions was adjusted with DCl or NaOD. The pD values were calculated according to Glasoe and Long [7]. Ionic strength was kept constant with tetraethylammonium chloride. The pH measurements were made with a Radiometer pH4 pH meter. The measurements in the presence of calcium chloride were stopped at pD 9.9 because of calcium hydroxide precipitation.

RESULTS

The ^{13}C-FT-NMR spectrum of taurine is shown in Fig. 1. The assignment of the peaks was made on the basis of chemical shift values and by comparison with literature data. The pD dependence of the chemical shift of both carbons is shown by the upper curves of Figs 2 and 3. The results obtained are consistent with the values of pK_a 1.5 and pK_b 9.08 reported in the literature [8]. With the addition of equimolar amounts of calcium chloride, a downfield shift of the carbon adjacent to the sulphonic group was observed at low pD values. This shift is

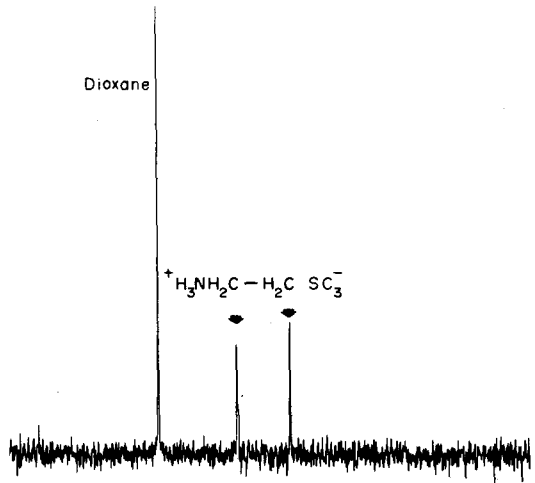

Fig. 1. ^{13}C FT-NMR spectrum of taurine (0.75 M) in D_2O, pD 7.4. pD calculated according to Glasoe and Long [7]. Dioxane was used as reference standard. Sweep width 6 Khz. Number of scans 5000.

explained by the decreased charge density following the interaction of the sulphonic group with calcium ions. The same effect can also explain the very little shift of the carbon near to the amino group, at pD values lower than 7.4. At lower pD values, in fact, the amino group is totally protonated, and cannot interact with positively charged calcium ions. At pD values higher than 7.4 the observed chemical shift of the carbon adjacent to the amino group in the presence of calcium is the result of a direct interaction with the deprotonate amino groups. This interpretation was confirmed by the use of Mn^{2+} as a paramagnetic probe, which has been shown to replace calcium ion without any loss of biochemical activity. At the temperature of the experiments, the presence of a little amount of Mn^3 ions such that the ratio metal to ligand is about 2×10^{-4}, induces an evaluable broadening of the NMR lines which can be discussed in terms of T_{2p}^{-1} defined as $T_{2M}^{-1}-T_{2O}^{-1}$ where T_{2M}^{-1} and T_{2O}^{-1} are the line widths with and without the metal in solution respectively. As discussed in various preceding papers [9–11], a greater T_{2p}^{-1}

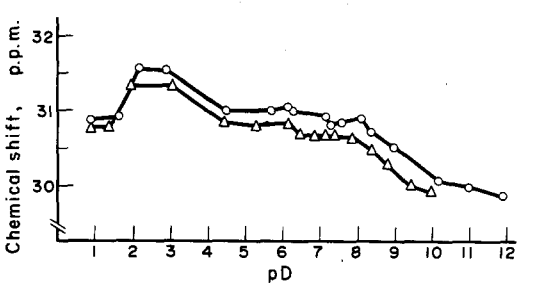

Fig. 2. pH dependence of the chemical shift of the carbon of taurine adjacent to the sulphonic group, in the presence (—△—) and in the absence of $CaCl_2$(—○—).

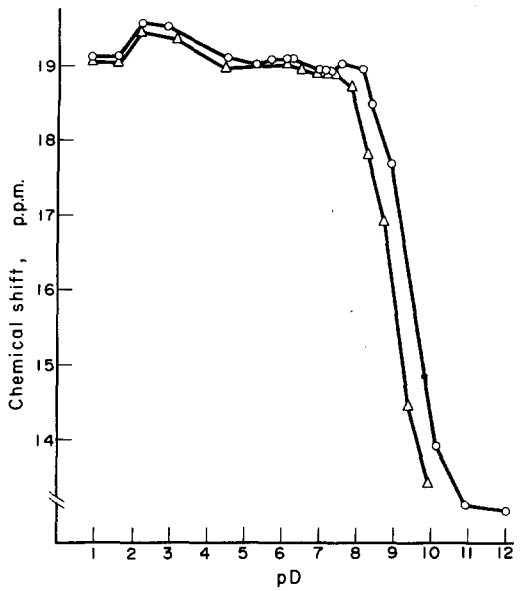

Fig. 3. pD dependence of the chemical shift of the carbon of taurine adjacent to the amino group, in the presence (—△—) and in the absence of $CaCl_2$ (—○—).

value can be related to a more direct interaction with the metal ion.

At pD 7.4 a T_{2p}^{-1} value of 3.46 sec^{-1} was measured for the carbon near to the sulphonic group, whereas a negligible contribution was evident for the carbon atom near to the amino group. When the pD was raised to 7.8 a T_{2p}^{-1} value of 7.95 sec^{-1} was measured for the C-NH_2 whereas the paramagnetic contribution T_{2p}^{-1} to the C-SO_3^- remained constant. These experimental findings point out that the aminogroup can interact with the metal ion in a sizeable way only at PD $\geq$ 7.8.

The formation constant for calcium–taurine complex, defined as $K = [ML]/[M^{2+}][L^-]$, $[L^-]$ and $[M^{2+}]$ being free ligand and metal respectively, were calculated with the method described by Rabénstein [12, 13]. A value of 2.23 $\pm$ 0.46 (S.E.) was obtained for the formation constant relative to the sulphonic group, and a value of 5.02 $\pm$ 1.51 (S.E.) for the amino group. The first formation constant found to be 0.99 by potentiometric techniques.*

DISCUSSION

The reported results demonstrate that a definite interaction occurs between taurine and calcium in aqueous solution. The observed constants of formation are anyway very low, much lower than those observed in the case of some aminoacids like glycine and alanine (3.7×10^2 and 5.8×10^2 respectively) [14]. The low value of taurine–calcium formation constants can probably explain the negative results obtained by Igisu *et al.* [5]. Moreover a chelate between calcium and taurine can be formed only at pH greater than 7. At lower pH the interaction is limited to the sulphonic group and is quantitatively less important. Despite the low formation constants, we think that a direct physico–chemical interaction between calcium and taurine can explain some of the results described in previous papers [1–4]. In fact the taurine content in cardiac cells is very high (39 mM) [2], and is in great excess relative to the calcium there in (2.24 mM) [15]. Using the formation constants we have obtained, and assuming that at the intracellular pH of 6.9 the calcium can be bound only to the sulphonic group, we can calculate that 0.185 mMoles/l of calcium, corresponding to 8.2 per cent of the total calcium, can be bound to taurine and so modify the intracellular calcium transfer. Obviously the extrapolation of the data obtained in simplified solutions to the actual situation observed in the cell is questionable. It seems anyway that the calculated affinity constants can justify some of the observations of modified calcium transfer rates in the presence of taurine.

* L. Paoletti and A. Vacca, preliminary results. Personal communication.

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