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Micelle Formation between 5-Hydroxytryptamine and Adenosine Triphosphate in Platelet Storage Organelles

Abstract. *As judged by analytical ultracentrifugation, 5-hydroxytryptamine and adenosine-5′-triphosphate form micelles in artificial mixtures and also in storage organelles containing 5-hydroxytryptamine of blood platelets of rabbits. Their average apparent molecular weights depend on the concentration and on the molar ratio of the two constituents. The 5-hydroxytryptamine and adenosine triphosphate of these 5-hydroxytryptamine organelles may be stored in vivo together as micelles with apparent molecular weights of several hundred thousands or more.*

In the blood platelets, 5-hydroxytryptamine (5HT) and adenosine-5′-triphosphate (ATP) are mainly localized in special intracellular organelles, which, in rabbits, also contain histamine (*1*). In these organelles the content of 5HT and ATP relative to their volume is very high, exceeding 20 percent (weight to volume) for 5HT and 25 percent (weight to volume) for ATP (*2*). These compounds, if present in a monomolecular solution or even if dissolved as an undissociated salt containing 2 or 3 moles of 5HT and 1 mole of ATP, would give rise to an osmotic pressure exceeding that of plasma, where the isolated organelles are relatively stable.

The involvement of ATP in the storage of 5HT by platelets has been discussed (*3*, *4*), and the formation of 5HT-ATP complexes of low molecular weight in vitro was postulated based on results of electrometric titration (*5*). Our experiments give evidence for micelle formation between 5HT and ATP in artificial mixtures as well as in organelles of blood platelets containing 5HT.

Apparent molecular weights were determined at 20°C by equilibrium centrifugation in a Spinco analytical ultracentrifuge equipped with schlieren optics; a 12-mm centerpiece was used for the 1 percent solution, and a 3-mm centerpiece was used for all other experiments. The solutions were layered on a cushion of silicone oil. After equilibrium was achieved, we determined apparent molecular weights from the concentration gradient (*6*) at three different spots in the cell, each representing different concentrations. Solutions of ATP and 5HT oxalate in 0.15*M* sodium-potassium-phosphate buffer (*p*H 7.2) were prepared. The 5HT organelles of blood platelets of rabbits were isolated by density-gradient centrifugation in Urografin (*7*). The isolated organelles were thoroughly washed with Tyrode buffer to remove the Urografin; they were then disrupted by freezing in 0.6 ml of water, and the membranes were centrifuged at 159,000*g*. The supernatant solution was evaporated under normal pressure at about 10°C in a stream of nitrogen to a volume of approximately 0.015 ml; the remaining solution was immediately subjected to analytical ultracentrifugation. In the two experiments, the organelles of 36 and of 69 rabbits were pooled, with final concentrations of 5HT plus ATP of 4 and 15 percent, respectively.

The dependence of micelle weight on concentration of solutions of 5HT and ATP in molar ratios of 2 to 1 is demonstrated in Fig. 1. In mixtures of 5HT plus ATP (curve 3), the apparent molecular weights are about an order of magnitude higher than those of the single solutes (curves 1 and 2). Self-association in water occurs in one-component systems, that is, between molecules of mixed hydrophilic-hydrophobic structure such as soaps (*8*), detergents (*9*), and organic dyes (*10*), and also between many proteins (insulin) (*11*) and aromatic bases such as purines (*12*). Association in mixtures (5HT plus ATP) may be substantially higher than in the solutions of the single compounds. A separation of a second liquid phase of high viscosity is obtained on cooling to 2°C a solution (30 percent by weight) of 5HT and ATP in a molar ratio of 2 to 1. Concentrations of 5HT and ATP up to 90 percent and more have been found in the separated phase, the molar ratio between 5HT and ATP being about 2.2. Evidently, formation of micelles between 5HT and ATP has occurred.

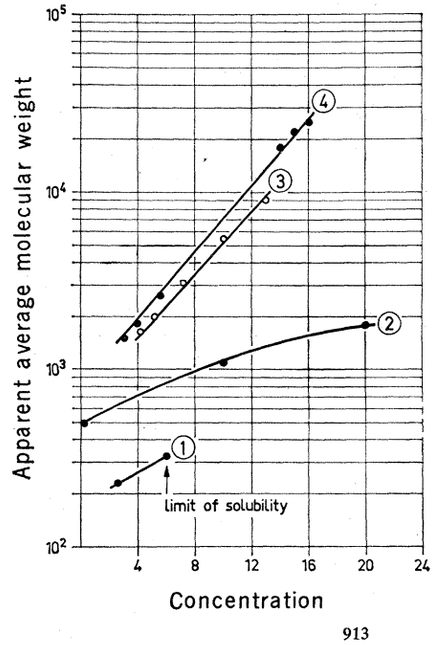

Fig. 1. Dependence of apparent molecular weight on concentration (percent by weight): Curve 1, 5HT oxalate; curve 2, ATP; curve 3, (circles), 5HT oxalate plus ATP, molar ratio 2 (*p*H about 2) and (square), 5HT chloride plus ATP, molar ratio 2 (*p*H adjusted to 6); curve 4, fluid from 5HT organelles (*p*H about 6).

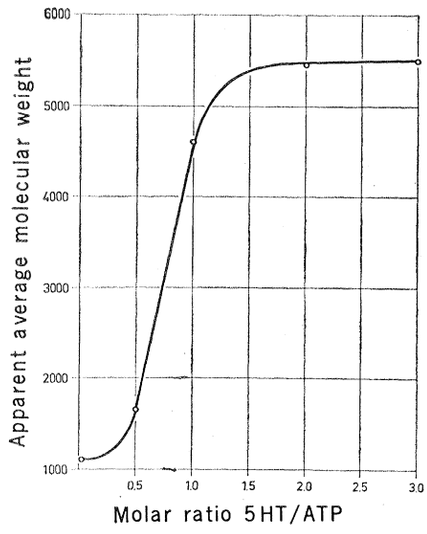

Fig. 2. Dependence of apparent molecular weight of mixtures of 5HT oxalate and ATP on their molar ratio at constant total concentration of the two solutes (10 percent by weight).

The solubility of 5HT is substantially increased, because that of 5HT oxalate used for the present experiments amounts only to about 6 percent.

The dependence of the apparent molecular weights of mixtures of 5HT and ATP on their molar ratio is presented in Fig. 2, the total concentration of the two solutes added together being 10 percent by weight. Evidently micelle formation is favored at molar ratios above 0.5 and reaches a plateau at a ratio of about 2 and more. Ratios higher than about 3 cannot be realized because of limited solubility of 5HT.

Micelles are also formed by constituents of the 5HT organelles (Fig. 1, curve 4), apparent molecular weights being even higher than those obtained with artificial mixtures of 2 moles of 5HT and 1 mole of ATP (Fig. 1, curve 3). Again a very pronounced concentration dependence of the apparent molecular weights is obvious. The following findings give evidence that these micelles are mainly composed of 5HT and ATP in a molar ratio of about 2. (i) 5HT and ATP are major constituents of the 5HT organelles, their molar ratio being about 2 to 3 (*7*). (ii) Artificial mixtures of 5HT and ATP in a molar ratio of 2 to 1 show very pronounced micelle formation (Fig. 1, curve 3), the concentration dependence of which is quite similar to that in the storage organelles of 5HT (Fig. 1, curve 4). Extrapolation of curve 4 in Fig. 1 to the concentration times 0 yields an apparent molecular weight of about 800 which is similar to that of a monomer consisting of one molecule of ATP and two molecules of 5HT (molecular weight, 859). (iii) The ultraviolet spectrum of the content of 5HT organelles corresponds to that of a mixture of 5HT and ATP in a molar ratio of about 2 (*13*).

The higher apparent molecular weights found with biological material as compared with those of artificial mixtures (compare curves 3 and 4 of Fig. 1) may be due to additional stabilizing factors. An influence of ions present and of temperature on micelle weight has been found in preliminary experiments.

Our results suggest that in the 5HT storage organelles in vivo, 5HT is fixed together with ATP in micelles. The micelle weights in the organelles may be very high since extrapolation of curve 4 in Fig. 2 to a concentration in vivo of about 45 percent 5HT plus ATP yields apparent molecular weights of several millions. Thus, micelle formation may explain the osmotic stability of the organelles. Micelles may be formed by vertical stacking of units of one ATP plus two to three 5HT molecules.

Micelle formation may be a general principle for the storage of compounds which form salts or complexes of mixed hydrophilic-hydrophobic character, such as aromatic monoamines plus ATP. We have evidence for the aggregation of catecholamines and ATP in the contents of granules of bovine adrenal medulla.

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Degradation and Disappearance of ortho, para Isomer of Technical DDT in Living and Dead Avian Tissues

Abstract. *The* o,p′*-DDT in technical DDT is broken down to* p,p′*-DDT and then to 1,1-dichloro-2,2-*bis(p-*chlorophenyl)ethylene in living avian tissue. In the anaerobic conditions existing after death,* o,p′*-DDT is metabolized to 1,1-dichloro-2-*(o-*chlorophenyl)-2-*(p-*chlorophenyl)ethane. The absence of* o,p′*-DDT and metabolites in field specimens is ascribed to the rapid rate of breakdown and a masking of the 1,1-dichloro-2-*(o-*chlorophenyl)-2-*(p-*chlorophenyl)ethane residue during analysis by the relatively large amounts of 1,1-dichloro-2,2-*bis(p-*chlorophenyl)-ethylene.*

In Britain a nationwide survey of organochlorine residues in wild birds since 1961 was conducted (*1–3*) and showed the presence of compounds derived from DDT in the majority of samples analyzed. Of these compounds the one most commonly found has been *p,p′*-DDE (*4*). This compound was found in 816 (97.1 percent) out of 840 predatory bird samples (livers and eggs) examined (*2*). The other commonly occurring compounds were *p,p′*-DDT (27.0 percent) and *p,p′*-DDD (27.0 percent). The residue of *p,p′*-DDT would result from consuming prey which had recently fed on food contaminated with technical DDT [*p,p′*-DDT forms 65 to 73 percent of technical DDT (*5*)]. The *p,p′*-DDE is the main metabolite produced on degradation of *p-p′*-DDT in the living avian body (*6, 7*). A small percentage [0.17 to 4.0 percent (*5*)] of *p,p′*-DDD occurs in technical DDT, and it is also used in small quantities as an insecticide (Rhothane). However, the most