A Possible Mechanism for Uptake of Biogenic Amines by Storage Organelles: Incorporation into Nucleotide-Metal Aggregates

Subcellular organelles storing monoamines, e.g. the 5-hydroxytryptamine (5HT) organelles of rabbit blood platelets, contain large amounts of nucleotides such as adenosine-5'-triphosphate (ATP) and guanosine-5'-triphosphate (GTP) as well as Ca and Mg[1-3]. The 5HT organelles take up and retain additional amounts of amine in vitro and in vivo[4,5]. Mixtures of these nucleotides with biogenic amines as well as the nucleotides alone form aggregates with Ca and Mg[6,8], while the amines alone do not aggregate with these metal ions[9].

This paper presents experimental evidence that the apparent molecular weights of nucleotide-metal aggregates markedly increase on addition of monoamines and that differences in this respect exist between various nucleotides.

Apparent average molecular weights were determined in the temperature range between 5 and 35 °C by equilibrium centrifugation in a Spinco analytical ultracentrifuge equipped with Schlieren optics according to the method of YPHANTIS[10] as previously described[3,6-8,11]. The calculations were based on the concentrations of nucleotides and biogenic amines disregarding that of the electrolytes. The temperature for phase separation was established by means of a water thermostat, the temperature of which was gradually decreased. The concentration of nucleotides and of biogenic amines was checked by spectrophotometric methods[12,13], and the content of alkaline-earth metal ions was determined by atomic absorption spectrometry. 12% w/v solutions of the disodium salts of ATP (Boehringer, Mannheim) and of GTP (Fluka, Buchs) and of the trisodium salt of uridine-5'-triphosphate (UTP; Fluka, Buchs) were used. Noradrenaline (Fluka, Buchs) was added as base. 5HT, available only as its oxalic acid salt (Fluka, Buchs) was liberated by precipitation of the oxalic acid with a stoichiometric amount of $Ca(OH)_2$. The final solutions were adjusted to pH 6 with $NaHCO_3$ or HCl, as this pH corresponds to that found in 5HT-storage organelles of rabbit blood platelets[7].

Figure 1 shows that addition of $MgCl_2$ and $CaCl_2$ to a 12% w/v solution of ATP causes an increase in the apparent average molecular weights (curve 2), probably due to formation of ATP aggregates[6]. The aggregation is enhanced with decreasing temperature but still noticeable at 35 °C. Molar ratios of Mg and Ca to ATP similar to those used in vitro (0.8 and 0.2 respectively) have been found in the 5HT organelles of rabbit platelets[3]. Addition of 5HT to the solutions which contain ATP, $MgCl_2$ and $CaCl_2$ further increases the apparent average molecular weights. Thereby 5HT in a molar ratio to ATP of 2:1 has a greater effect than in a molar ratio of only 1 (curve 3 and 4). A second liquid phase is separated at 13 °C, probably due to the formation of aggregates of very large size. The molar ratio of 5HT to ATP of 2 corresponds approximately to that found in the 5HT storage organelles of rabbit blood platelets[1]. The concentration of ATP (w/v) in the organelles is however about twice as high (approximately 25%)[1,14] as that chosen in the described aqueous solutions (12%). Therefore, in the organelles an even more marked aggregation may be expected to occur than in the artificial system, since aggregation has been shown to increase markedly with rising concentration of the nucleotides[7].

Figure 2 presents the apparent molecular weights at 20 °C after addition of increasing amounts of noradrenaline (NA) to 12% w/v solutions of various nucleotides in the presence of $CaCl_2$ (molar ratio $CaCl_2$ to nucleotides 0.23). The rise in the apparent molecular weights is most pronounced with GTP (curve 1). A second liquid phase separates at a molar ratio of NA to GTP of about 4. A marked increase in the molecular weights on addition of NA is also observed with ATP (curve 2). A maximum is reached at molar ratios of NA to nucleotide of 4–5. At this molar ratio a second liquid phase separates, when the solutions are cooled to about 9 °C. The decrease in the average apparent molecular weights at ratios superior to 5 (curve 2) is probably due to the presence of excess amounts of unaggregated NA. UTP in contrast to GTP and ATP shows only moderate increases in apparent molecular weights on addition of NA (curve 3). In this connection it is of interest that molar ratios of catecholamines to ATP of about 4 have been determined in chromaffin granules of adrenal medulla[15] and that 5HT storage organelles of blood platelets as well as the chromaffin granules contain in the main ATP and GTP but little UTP[1,2,15,16]

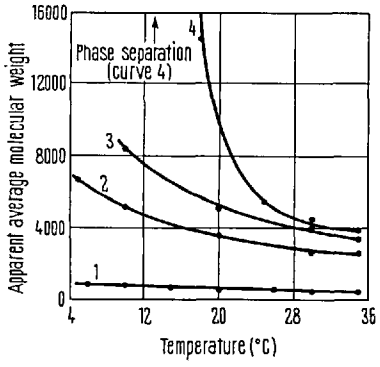

Fig. 1. Apparent average molecular weights of a 12% w/v aqueous solution of adenosine-5'-triphosphate (ATP). Curve 1: Without additives. Curve 2: Addition of $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 0.8 and 0.2, respectively. Curve 3: Addition of 5-hydroxytryptamine (5HT), $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 1, 0.8 and 0.2, respectively. Curve 4: Addition of 5HT, $MgCl_2$ and $CaCl_2$, molar ratios to ATP: 2, 0.8 and 0.2, respectively. The pH was adjusted to 6.

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The second phases which separate from solutions containing amines, nucleotides and bivalent cations are highly viscous, transparent and located at the bottom of the tube[11]. They contain all 3 components in concentrations several times higher than in the supernatant.

The results of analytical ultracentrifugation as well as the composition of the second phases indicate that addition of amines to nucleotide-metal aggregates enhances their average apparent molecular weight and that the new aggregates consist of nucleotides, amines and bivalent cations. Therefore, nucleotide-metal aggregates seem to be able to incorporate added monoamines.

Based on these experimental results and on the previous findings that amines in contrast to the nucleotides do not aggregate in the presence of Ca^{++} and Mg^{++} a tentative hypothesis for the uptake of biogenic monoamines by storage organelles may be presented. Accordingly the organelles which have a high concentration of bivalent cations would primarily accumulate nucleotides in the form of aggregates. These aggregates can probably not leave the organelles, as they are too large to pass through the surrounding membrane. The amines, e.g. the 5HT of blood platelets, have been shown to enter the cells by an active transport at the level of the cytoplasmic membrane[17] and also to be able to penetrate into the organelles[18]. Here, they are probably incorporated into the preexisting nucleotide-metal aggregates. As a consequence, the concentration gradient of the free amine between exterior and interior of the organelles would be maintained, enabling more of the amine to penetrate and to be incorporated into nucleotide-metal aggregates. This may result in an accumulation of amines in the organelles without the anticipation of an active transport mechanism for amines at the level of the membrane of the organelles[14,18].

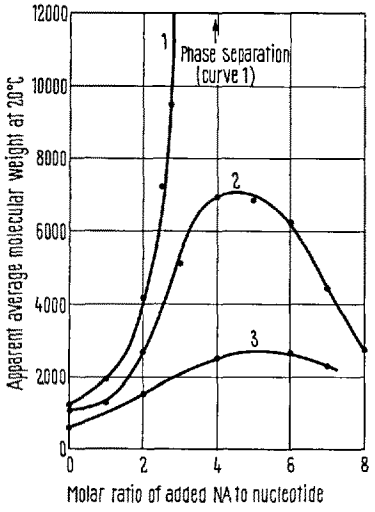

Fig. 2. Effect of added noradrenaline (NA) on the apparent average molecular weight at 20 °C of 12% w/v solutions of different nucleotides, containing $CaCl_2$ in a molar ratio to the nucleotide of 0.23; pH adjusted to 6. Curve 1: Guanosine-5'-triphosphate (GTP). Curve 2: Adenosine-5'-triphosphate (ATP). Curve 3: Uridine-5'-triphosphate (UTP).

This hypothesis is supported by the finding that in platelets of guinea-pig, which normally are poor in 5HT, the isolated storage organelles contain nucleotides and bivalent metals in high concentrations but only little 5HT[19]. On exposure of these platelets to exogenous 5HT the organelles take up and store considerable amounts of the amine[4].

In conclusion, preexisting aggregates between ATP or GTP and bivalent cations seem to be able to incorporate biogenic monoamines, resulting in a further increase in the apparent molecular weight. This may be an essential mechanism for the uptake and storage of amines in the subcellular organelles, e.g. of blood platelets, adrenal medulla and possibly sympathetic nerve endings.

Zusammenfassung. Analytische Ultrazentrifugation ergibt, dass Adenosin-5'-triphosphat und Guanosin-5'-triphosphat in Gegenwart von Ca und Mg hochmolekulare Aggregate bilden. Die scheinbaren mittleren Molekulargewichte dieser Aggregate nehmen nach Zusatz von 5-Hydroxytryptamin oder Noradrenalin stark zu. Es wird daraus geschlossen, dass Nucleotidaggregate biogene Amine inkorporieren, wobei Nucleotid-Aminaggregate entstehen. Dies erklärt möglicherweise die Aufnahme und Speicherung von biogenen Aminen in nucleotidhaltigen subzellulären Organellen, z.B. von Blutplättchen, Nebennierenmark und noradrenergen Nerven.

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Relationship Between Rm Values and Biological Activity of Steroid Esters

A reversed-phase TLC method was previously used in order to determine the Rm values of antibiotics and testosterone derivatives as an expression of their lipophilic character[1-3]. A significant relationship was observed between the Rm values, which were also shown to be very well correlated with the Hansch' π values[3], and the hemolytic activity of testosterone compounds[3]. The purpose of the present paper was to show that the Rm values are also correlated with the in vivo activity of long-acting testosterone esters.

The Rm values of testosterone esters were previously determined in the presence of acetone or methanol in the mobile phase[3]. It was shown that, because of a linear relationship, both series of Rm values were equally correlated with biological activity[3]. The Rm values at 54% acetone in the mobile phase were used in the present work (Table). Higher and/or positive Rm values indicate compounds more lipophilic than those characterized by lower and/or negative Rm values.

Some activity data were taken from the literature[4]; the biological response (BR) was represented by the time (days) of maximum effect of testosterone esters in the capon's comb test[5] (Table).

The relationship between lipophilic character (Rm values) and logarithm of the biological response (log BR values) was analyzed by means of regression analysis. A