THE INTERACTION OF BIOGENIC AMINES WITH ADENOSINE-5′-TRIPHOSPHATE: A CALORIMETRIC STUDY

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1. Introduction

Although it is generally accepted that the function of the storage vesicles in monoaminergic nerve terminals is uptake, storage and release of biogenic amines, as well as potential transmitters [1, 2], most of the individual processes are still not fully elucidated [3]. By uptake and storage the transmitters are inactivated and prevented from biochemical degradation. These processes are necessary to form a pool of transmitters for neuronal transmission. It is possible to study the mentioned processes with storage vesicles, generally isolated from adrenal medulla and blood platelets. In general they contain large amounts of monoamines, nucleotides, especially adenosine-5′-triphosphate (ATP), bivalent cations and in the case of chromaffin granules some soluble proteins as chromogranins and dopamine-β-hydroxylase.

The bovine medullary storage granules have concentrations of catecholamines (adrenaline and noradrenaline) and ATP as high as 0.6 M (10% w/v) and 0.15 M (7.5% w/v) respectively [4, 5]. Organelles, storing 5-hydroxytryptamine (5-HT) isolated from rabbit blood platelets contain about 1.1 M (20% w/v) 5-HT and 0.5 M (25% w/v) ATP [6, 7]. The corresponding osmolarity is at least twice as high as the one of mammalian body fluids. Consequently those vesicles could hardly be osmotically stable if the amines and ATP are present as monomers. Hillarp [7] therefore suggested a storage complex between the amines and ATP which could account for the stability of these vesicles. Several studies [8] with different methods (e.g. ultracentrifugation, nuclear magnetic resonance, infrared and fluorescence spectroscopy) have been carried out to confirm this hypothesis. To get further information about the mechanism of interaction between biogenic amines and ATP we performed microcalorimetric experiments.

2. Material and methods

The biogenic amines L-adrenaline, L-noradrenaline–hydrochloride, 3-hydroxytyramine–hydrochloride (dopamine), 5-hydroxytryptamine–hydrogen–maleinate, histamine–dihydrochloride, tyramine–hydrochloride and acetylcholine–jodide were purchased from Fluka Ag, Buchs and adenosine-5′-triphosphate–disodiumsalt·$3H_2O$ (ATP) from Boehringer Mannheim. All other chemicals were of analytical purity.

The calorimetric measurements were performed in 0.066 M phosphate buffer pH 5.4–6.8 with a flow microcalorimeter LKB 10700/1 at 25°C. The flow rates of both reactant solutions were 5.56 ± 0.05 μl/sec. A continuous pH control of all solutions and reactant mixtures was carried out with an Orion model 701 digital pH-meter.

3. Results and discussion

We measured the heat of dilution of ATP solutions in the concentration range 1.65×10^{-2} to 1.98×10^{-1} M ATP (1–12% ATP). The corresponding ΔH-values show a concentration dependency with a saturation value of 1.8 ± 0.1 kcal/mole at a concentration of about 2×10^{-1} M ATP; but no pH-effect could be detected (pH 5.4–6.8). From these results a weak self-association of ATP can be concluded, which is in good agreement with ultracentrifugation data [9–11].

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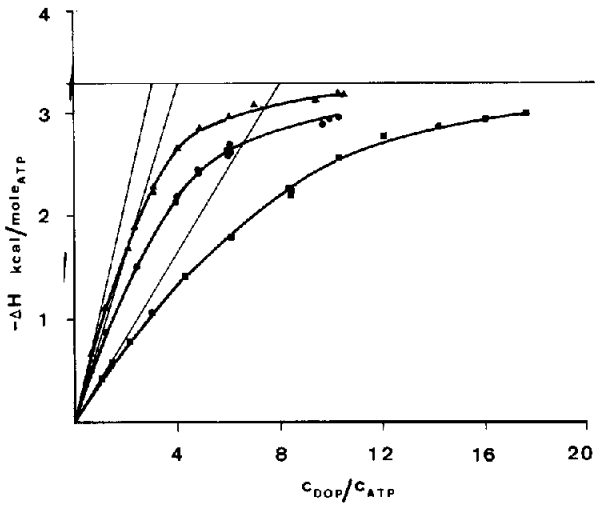

Fig. 1. Corrected experimental heats of reaction per mole ATP as a function of the molar ratio dopamine (DOP)/ATP in 0.066 M phosphate buffer, pH 6.8 at 25°C and constant ATP concentration: (■) c_{ATP} = 0.0165 M; (●) c_{ATP} = 0.041 M; (▲) c_{ATP} = 0.119 M.

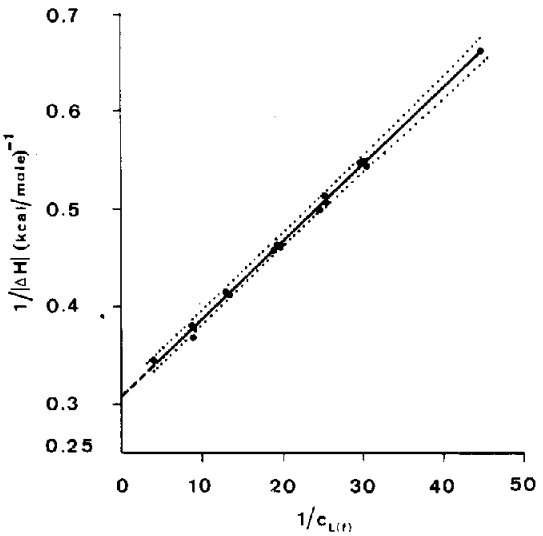

Fig. 2. Double reciprocal plot of the system dopamine-ATP in 0.066 M phosphate buffer, pH 7.6 at 25°C. ($c_{L(f)}$ = concentration of free dopamine at equilibrium).

The interaction of the biogenic amines with ATP was studied at constant ATP concentration (1.65×10^{-2}, 4.1×10^{-2}, 1.19×10^{-1}), due to the fact that in isolated chromaffine granules, the molar ratio amine/ATP has been found to be about 4:1, whereas the amine concentration was varied over a wide range to get a saturation curve. This enables us to elucidate [12] the ΔH°-values per mole ATP; the association constant K and the stoichiometry with the assumption that only equivalent binding sites are involved. Fig. 1 shows the corrected heats of reaction per mole ATP as a function of the molar ratio of dopamine/ATP. It is evident that the stoichiometry of the dopamine–ATP-complex is concentration dependent and decreases with increasing ATP concentration. This phenomenon may be referred to the stacking of ATP molecules. The corresponding ΔH°-values for the three different ATP concentrations studied are constant with the resulting average of -3.30 ± 0.14 kcal/mole ATP. Similar results were obtained with adrenaline, noradrenaline, histamine and tyramine.

For solubility reasons an evaluation of the ΔH°-values for the interaction of ATP with 5-HT was not possible. 5-HT seems to have a very high potency to aggregate with the nucleotide compared with the other amines studies (see also table 2). The determination of the association constants [13] for the amine–ATP complexes was carried out for an ATP concentration of 4.1×10^{-2} M. Fig. 2 shows the double reciprocal plot with linear regression analysis and confidential limits (dotted lines). The value of the correlation coefficient was determined as 0.99. For an assumed 4:1 complex of dopamine to ATP the calculated value of the association constant is about 40 M^{-1} per binding site. It is important to note that acetylcholine does not react with ATP; this different behaviour of cholinergic and adrenergic transmitters could be of great interest.

The ΔH°-values in table 1 were calculated from theoretical saturation concentrations; their biological relevance should therefore not be overestimated. Due to this fact we measured the enthalpy change resulting from the interaction of the amines with ATP and ATP-Mg^{2+} under conditions corresponding to those of storage granules of adrenal medulla. The results are summarized in table 2. Furthermore in preliminary experiments the 'potential heat of dissociation' of the amine–ATP complex in a biological system was measured. This was performed directly in the calorimeter by mixing intact granules of bovine adrenal medulla with bidistilled water. Surprisingly the found ΔH-values of $+2.9 \pm 0.3$ kcal/mole ATP are

Table 1
Thermodynamic parameters of the interaction of biogenic amines with ATP in 0.066 M phosphate buffer, pH 6.8 at 25° C.

Amine	K (M^{-1})	$-\Delta G^{\circ}$ (kcal/mole)	$-\Delta H^{\circ}$ (kcal/mole)	$-\Delta S$ (e.u)
L-Adrenaline*	36.2	2.13	2.54	1.4
L-Noradrenaline	67.6	2.50	3.45	3.2
Dopamine	38.7	2.17	3.26	3.7
Histamine	106.0	2.76	6.63	13.0
Tyramine	37.5	2.15	3.16	3.4
Acetylcholine	0	0	0	0

* pH 5.6.

of the same order as those of our artificial systems. The above findings indicate a decreasing potency of agregation with ATP for the amines studied in the order: 5-hydroxytryptamine, noradrenaline, dopamine, adrenaline, tyramine, histamine, which is in a good agreement with ultracentrifugation data [9–11] of artificial solutions.

From the described microcalorimetric studies and further spectroscopic data [14–16] the following binding model can be derived: catecholamines, 5-HT, histamine, as well as tyramine are able to form weak complexes with ATP, which is evident from the very low dissociation constant. The fact that acetylcholine does not interact with ATP allows to postulate that the indole- or catecholamine ring is important for complex formation. This could explain the relatively high molecular weights determined by ultracentrifugation studies. The values of thermodynamic parameters confirm the participation of stabilizing electrostatic forces between the positively charged nitrogen of the amines and the negatively charged phosphate groups of the nucleotides. The complex between biogenic amines and ATP seems to be of extremely dynamic nature, which could explain not only the osmotic stability, as proposed by Hillarp [7], but nevertheless does not prevent a rapid release of neurotransmitters when triggered by a stimulus.

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Table 2
Heats of reaction of artificial ATP-amine and ATP/Mg^{2+}-amine systems at 25°C.

Amine (X)	pH	ATP–(X) $-\Delta H^{*}$ (kcal/mole$_{ATP}$)	ATP $-\Delta H^{*}$ (kcal/mole$_{ATP}$)
L-Adrenaline	5.4	2.55	2.27
L-Noradrenaline	5.4	3.46	3.21
Dopamine	6.8	3.07	2.78
Tyramine	6.8	2.33	2.01
Histamine	6.8	2.84	1.97
5-hydroxy-tryptamine	6.8	3.89	3.38
Acetylcholine	6.8	(0.01)	0

* ± 4.2%.

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