CALORIMETRIC INVESTIGATION OF 5-METHOXYTRYPTAMINE BINDING TO DNA AND POLY(A)

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Received 13 September 1976

1. Introduction

It is well known that many aromatic molecules, e.g. acridine dyes, are able to stack with purine and pyrimidine bases in single stranded and double stranded nucleic acids [1–3]. The possibility that such a type of complex involving aromatic amino acids may play a role in the interaction between nucleics and proteins has been recently investigated by several groups. Particularly it has been demonstrated that this type of complex takes place with model compounds involving derivatives of aromatic acids such as amines [4–7] or oligopeptides [8–16].

Whereas the enthalpy and entropy changes corresponding to the formation of stacked between several dyes and DNA are known [17], similar data for the complexes formed by aromatic amino acid derivatives and nucleic acids have not been reported yet. The knowledge of such values is necessary for a better description of the interaction phenomenon. We have recently determined, using circular dichroism measurements, the thermodynamic parameters for the binding amine derivative of tryptophan, 5-methoxytryptamine, to single stranded poly(A) [16] and to DNA [18]. In this paper results are reported from direct calorimetric measurements on the binding of 5-methoxytryptamine to poly(A) and DNA.

2. Experimental

Poly(A) was purchased from Miles Laboratory. Calf thymus DNA, a Sigma product, was treated with RNAase A and T_1 to remove traces of RNA. Residual proteins were removed by phenol treatment and precipitation of the DNA with ethanol and isopropanol. 5-methoxytryptamine was obtained from Fluka.

Solutions were prepared in the following buffer: sodium cacodylate (1 mM), sodium chloride (1 mM) and 0.2 mM EDTA, pH 7.0. To adjust this low ionic strength poly(A) and DNA were extensively dialyzed against this buffer in the cold.

Calorimetric measurements were performed using a batch microcalorimeter LKB equipped with glass cells. 3 ml of nucleic acid solution (4.6×10^{-4} M for poly(A) and 1.5×10^{-3} M for DNA) were mixed with a 1.5 ml sample of 5-methoxytryptamine. Heat quantities ranging from 50–300 mcal were measured. At the concentrations used in this study, neither the DNA nor the poly(A) exhibit measurable heat of dilution. To obtain only the heat of reaction the very weak heat of dilution of 5-methoxytryptamine was substracted. Alternatively direct substraction was made by filling the reference cells with buffer and 5-methoxytryptamine. At pH 7.0, 5-methoxytryptamine is entirely in the cationic form (the amino group has a pK of 9.0) and there is no exchange between the proton of the amine and the buffer during the binding, which could contribute to the heat observed during the reaction. It was checked that no pH change occurred during the mixing of the compound.

3. Results and discussion

Figure 1 shows the experimental heat of binding of 5-methoxytryptamine to DNA at 25°C as a function

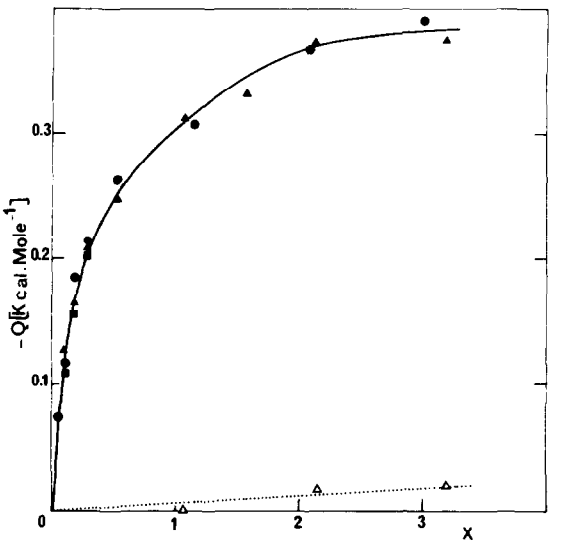

Fig.1. Heat of binding of 5-methoxytryptamine to Calf Thymus DNA in Kcal per mole of phosphate (——); X is the final ratio between the molecular concentration of amine and DNA. The concentration of DNA was constant and equal to 10^{-3} M. Symbols (● ■ ▲) correspond to different series of experiments. Heat of dilution of 5-methoxytryptamine (△ △). Buffer: 1 mM NaCl, 1 mM sodium cacodylate, 2×10^{-4} M EDTA, pH 7.0; 25°C.

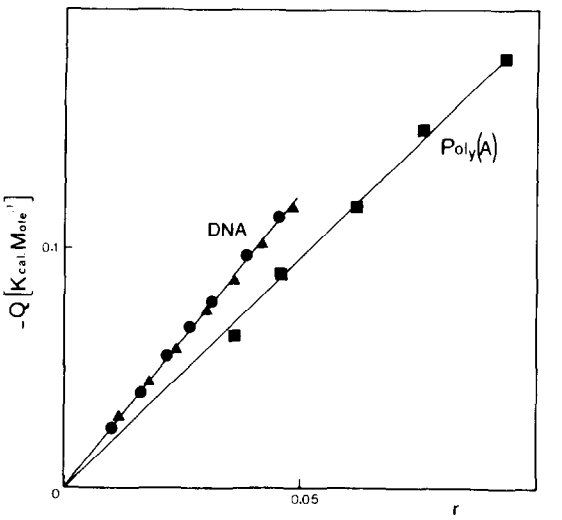

Fig.2. Heat of binding of 5-methoxytryptamine to Calf Thymus DNA and poly(A) as a function of the degree of saturation (r) at pH 7.0. (●) DNA (10^{-3} M) at 40°C; (▲) DNA (10^{-3} M) at 25°C, (■) poly(A) (3×10^{-4} M) at 25°C.

of the ratio X between the final concentrations of amine and DNA. The heat evolved in the reaction follows a classical saturation curve and for values of X larger than 2, only the dilution of the amine contributes to the experimental heat.

The degree of saturation (r) of DNA and poly(A) was measured by circular dichroism under conditions similar to those of the calorimetric experiments [16]. Unfortunately this technique allows only the determination of small values of r ($r < 0.05$ with DNA and $r < 0.1$ with poly(A)). A linear relation is obtained between the heat evolved during the reaction and the number of amine bound per phosphate (figure 2). The slope of this linear dependance yields the value of the binding enthalpy for low degrees of binding (r). Knowledge of the binding constants [16,18] allows the calculation of entropy changes during the reaction (table 1).

It was previously shown that 5-methoxytryptamine forms two types of complexes with poly(A), [15,16] and with DNA [18]: a purely electrostatic complex in which the positively charged NH_3^+ group of the amine interacts with the negatively charged phosphate group of the nucleic acid, and another complex in which a stacking interaction between the indole ring of 5-methoxytryptamine and the aromatic ring of nucleic acid bases is superimposed to the electrostatic interaction. Proton magnetic resonance measurements provided clear evidence for this stacking interaction both in poly(A) [4] and DNA [10] complexes. In both

Table 1
Thermodynamic parameters for the binding of 5-methoxtryptamine on Calf Thymus DNA and poly(A)

	T(°C)	$K \times 10^{-4}$ M^{-1}	ΔH (Kcal M^{-1})	ΔS (cal M^{-1} K^{-1})
DNA	25	2	−2.45	11
	40	1.6	−2.5	11
Poly(A)	25	0.5	−2	22

the poly(A) and DNA cases the second type of complex represents about 80% of the fixed amino molecules [15–18]. The enthalpy variation results from two terms: one which corresponds to the electrostatic binding and another one corresponding to the stacking of the aromatic rings. The first contribution appears in both types of complexes whereas the second one involves only 80% of the bound amines. It is not possible to obtain direct measurements of the enthalpy associated with the first type of complex since no quantitative data are known on the binding of compound giving only this type of complex, e.g., isopropylamine. Nevertheless it has been reported that the electrostatic binding enthalpy of polycations like spermine, tri-L-lysine, poly-L-lysine, tetra-L-lysine and Mg^{2+} are of the order of few hundred calories per mole of phosphate [19,20]. It seems reasonable to assume that the enthalpy variation corresponding to the electrostatic interaction between 5-methoxytryptamine and DNA might be of the same order of magnitude. Thus the enthalpy variation obtained in our study should be due to a large extent, to the stacking interaction. The observed low values of ΔH (table 1), seems to imply that the stacking enthalpy of a DNA base with the aromatic ring of 5-methoxytryptamine is of the same order of magnitude as the stacking enthalpy for two adjacent bases. The same conclusion holds in the case of poly(A).

Values between −5 and −8 Kcal/mol have been reported for the stacking of base in poly(A) [21–23]. In the case of DNA no similar data exist since hydrogen bonds also contribute to the stability of the helix, but one can think that the stacking enthalpy between the base is similar to that of poly(A). It should be noted that the formation of 5-methoxytryptamine-DNA and 5-methoxytryptamine-poly(A) complexes corresponds to quite similar reaction enthalpies. That this value is similar for single stranded and double stranded nucleic acids may be explained by two hypotheses:

(a) The stacking enthalpy between the ring of 5-methoxytryptamine and the bases of DNA or poly(A) are quite similar and there is no breaking of hydrogen bonds of DNA base pairs when 5-methoxytryptamine binds to DNA.

(b) The hydrogen bonds between the DNA bases belonging to the interaction site of DNA are broken and the resulting positive enthalpy variation is compensated by a stronger interaction of bases with the indole ring than in the case of poly(A).

It has been pointed out that in all cases where the intercalation of a dye in DNA has been documented, the calorimetric measurements lead to enthalpy values between −5 and −7 Kcal/mol of bound ligand [17]. The reaction enthalpy for proflavine binding to single stranded poly(A) was found to be around −8 Kcal/mol [24]. These values are by a larger than those found for the interaction of 5-methoxytryptamine with poly(A) and DNA. This may be due to the smaller size of the indole ring when compared to that of aromatic dyes, which leads to a smaller stacking interaction energy especially in the case of double stranded DNA where stacking could involve only one strand (the size of the indole ring is similar to that of purine base). This may also reflect the fact that the aromatic ring is only partially intercalated.

The entropy variation corresponding to the interaction of 5-methoxytryptamine with poly(A) is larger than that with DNA. The increase in entropy for an association reaction in aqueous solution has an important contribution from release of water molecules from the hydration layers of reacting molecules. The electrostatic interaction between the NH_3^+ group of 5-methoxytryptamine and the phosphate group as well as the stacking interaction might give rise to such a displacement of water molecules. The formation of complexes might also modify the conformation of the nucleic acid and of the amine. In the case of poly(A), the addition of 5-methoxytryptamine results in a destacking of the bases, thus an important disordering of the polynucleotide [7]. In the case of DNA we know that there is a conformational change of the DNA but we do not know what type of conformational change is involved and how this contributes to enthalpy variation. This makes it difficult to estimate the relative importance of the two types of contributions (hydration and structural changes) to the observed entropy variations.

There is almost no change between the thermodynamic parameters determined at 25 and 45°C in the case of DNA (figure 1 and table 1). Thus the variation of calorific capacity $C_p = d(\Delta H)/dT$ is zero, indicating that the structure of the complex does not change between these two temperatures, in agreement with previous results [13].

Aromatic amino acids could play a special role in the formation of protein acid complexes due to the direct interaction between the bases and the aromatic ring. The calorimetric data presented above should contribute to a better understanding of the thermodynamics of the type of interaction.

Acknowledgements

This work has been supported by contract No. 74-7-0359 with the D.G.R.S.T. (A.C.C. Interactions moleculaires in biologie).

References

[1] Lerman, L. S. (1963) Proc. Natl. Acad. Sci. USA 49, 94–102.
[2] Pritchard, N. J., Blake, A. and Peacoke, A. R. (1966) Nature (London) 212, 1360–1361.
[3] Dourlent, M. and Helene, C. (1971) Eur. J. Biochem. 23, 86–95.
[4] Helene, C., Dimicoli, J. L. and Brun, F. (1971) Biochemistry 10, 3802–3809.
[5] Helene, C. (1971) Nature 234, 120–121.
[6] Helene, C., Montenay-Garestier, T. and Dimicoli, J. L. (1971) Biochim. Biophys. Acta 254, 349–365.
[7] Durand, M., Borazan, H. N., Maurizot, J. C., Dimicoli, J. L. and Helene, C. (1976) Biochimie 58, 395–402.
[8] Brown, P. E. (1970) Biochim. Biophys. Acta 213, 282–287.
[9] Helene, C. and Dimicoli, J. L. (1972) FEBS Lett. 26, 6–10.
[10] Gabbay, E. J., Sanford, K. and Baxter, C. S. (1972) Biochemistry 11, 3429–3435.
[11] Gabbay, E. J., Sanford, K., Baxter, C. S. and Kapicak, L. (1973) Biochemistry 12, 4021–4029.
[12] Novak, R. L. and Donhal, J. (1973) Nature New Biol. 243, 155–157.
[13] Dimicoli, J. L. and Helene, C. (1974) Biochemistry 13, 714–723.
[14] Dimicoli, J. L. and Helene, C. (1974) Biochemistry 13, 724–730.
[15] Brun, F., Toulme, J. J. and Helene, C. (1975) Biochemistry 14, 558–563.
[16] Durand, M., Maurizot, J. C., Borazan, H. N. and Helene, C. (1975) Biochemistry 14, 563–569.
[17] Quadrifoglio, F., Crescenzi, V. and Giancotti, V. (1974) Biophys. Chem. 1, 319–324.
[18] Durand, M. (1976) Thesis, University of Orleans.
[19] Ross, P. D. and Shapiro, J. T. (1974) Biopolymers 13, 415–416.
[20] Giancotti, V., Cesaro, A. and V. Crescenzi, V. (1975) Biopolymers 14, 675–677.
[21] Van Holde, K. E., Brahms, J. and Michelson, A. M. (1965) J. Mol. Biol. 12, 726–739.
[22] Leng, M. and Felsenfeld, G. (1966) J. Mol. Biol. 15, 455–466.
[23] Neumann, E. and Ackermann, T. (1969) J. Phys. Chem. 73, 2170–2178.
[24] Hogrel, J. F. (1976) Thesis, University of Orleans.