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MICROCALORIMETRIC STUDY OF GLUTAMINE FIXATION ON THE GLUTAMINE-BINDING PROTEIN OF ESCHERICHIA COLI

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SUMMARY

The enthalpy variation (ΔH) induced by addition of glutamine to glutamine binding protein isolated from E.coli has been studied by microcalorimetry. The reaction was very exothermic.The free energy variation (ΔG) was calculated from the dissociation constant (K_D) measured by dialysis techniques. The entropic variation (ΔS) was deduced from ΔG and ΔH values ; it was found highly negative,indicating that an important conformational change is occuring. Comparison with others binding proteins and possible significance of such a phenomenon is discussed.

INTRODUCTION

Concerning gram negative bacteria, one of the outstanding questions about the periplasmic binding proteins involved in transport is the exact mode by which they do transfer their ligand across cell membrane (1,2,3). Several investigators assumed that a direct and specific interaction with the cytoplasmic membrane is a necessary step. Some authors did suggest that such an interaction may be preceded by a substrate induced conformational change of the binding protein creating thus the right stereochemistry necessary for the interaction. Boos (4) and Singer (5) recently reviewed this subject.

Evidence in support of such conformational changes has been presented in a few cases : Galactose (6,7), Maltose (8) and Glutamine (9,10) binding proteins of E.coli ; Sulfate (11) and Histidine (12) binding proteins of S. typhimurium . These studies have been done utilizing several methods like fluorescence,optical rotatory dispersion,I . R spectroscopy, gel electrophoresis, denaturants and N.M.R.

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Recently we studied binding of ligands on the leucine, isoleucine , valine binding protein (LIV-BP) of E.coli by the mean of microcalorimetry (13). In the present paper we report results obtained during the same kind of study that was done upon glutamine binding protein (Gln-BP) of E.coli. We measured heats of binding and affinity constant, parameters that permit a thermodynamical description of the binding reaction. The results obtained support the hypothesis that a strong conformational change occurs upon the binding reaction.

MATERIAL AND METHODS

E.coli : Strain Gln P_1 was a kind gift of D. Léon A. Heppel. All cultures were grown in a synthetic medium described previously by Tanaka, Lerner and Lin (14) supplemented with 1% sodium succinate.

Chemicals : Isotopic material (L G-^{3}H, Glutamine, L U-^{14}C Glutamine) was obtained from the Radiochemical centre Amersham. Non-radioactive L + Glutamine was purchased from Fluka. DEAE cellulose was from Whatman, Ultrogel from L.K.B. and scintillation liquid from Lumac.
All others chemicals were obtained from Prolabo and Merck.

Protein preparation :

The Gln-BP has been purified to homogeneity by a method derived from the one published by Wiener and Heppel (9). One hundred grams (wet weight) obtained from a 16 liters culture (Chemap fermentor) were osmotically shocked. The four liters crud shock fluid was concentrated by ultrafiltration throught an Amicon UM 10 membrane.
The filtrate, after extensive dialysis against 10 mM Tris HCl pH 7,4 buffer, was applied on a column (26x700mm) of DEAE cellulose. The Gln-BP was not retained and appeared ahead in the flow-through fraction. This material was then concentrated and chromatographed on an Ultrogel ACA 4/4 column (26x1000 mm) equilibrated with 10 mM pH 7 phosphate buffer containing 0,1M NaCl and 5 mM sodium azide. The Gln-BP was then denatured by dialysis against an Urêa 8 M solution and renatured by dialysis against the same buffer as indicated above.

Binding assays :

The glutamine binding activity was determined by two different methods : equilibrium dialysis and rapid dialysis.
Equilibrium dialysis was carried out in Lucite chambers at 25°C as previously described by Furlong and Wiener (15) and by Wiener and Heppel (9).
Rapid dialysis was done according to the technique of Colowick and Womack (16).

Calorimetric Measurements :

Heats of binding of glutamine to Gln-BP were measured at 25 $\pm$ 0,1°C using an LKB batch calorimeter 10700-2- and an LKB flow calorimeter 10700-1-. In both cases, a Keithley 150 B microvolt ammeter coupled to a Sefram recorder were used to amplify and record output from the calorimeter. The electrical calibration heaters were checked by measuring the precision of the heat of neutralisation of HCl 3.10^{-4}N.

All binding experiments were performed using the 10 μV output of the amplifier. Concerning the batch calorimeter, all heats of reaction, substrate and protein dilution were determined in separate experiments in order to avoid errors due to differences in response of the thermopiles in each cell. Wet glass cells were used for all experimental work. Final volume in each cell was 6 ml. In the case of the flow calorimeter, pumps speed was adjusted to 10 ml per hour For all binding experiments the protein pulse volume was precisely fixed to 1 ml.

Protein concentration was determined by the method of Lowry et al (17).

RESULTS

The thermograms obtained during the binding reaction are shown in figure I.

Binding of glutamine to Gln BP is a strongly exothermic process. Heat values have been measured upon protein solutions of varied concentrations.(Table I). All the results are in good accordance and the heats of protein and substrate dilution are negligible.

According to the work of Amanuma et al (18) and to a recent study we did (19) the Gln-BP was treated by urea 8 M and then renatured, as indicated in methods, in order to release all the glutamine retained by the protein during purification. Then the Gln-BP was checked for its binding capacity by equilibrium and /or rapid dialysis. Experimental conditions were the same as those employed in the calorimetric cell. The value obtained for K_D is of the same order as those encountered in the litterature.

From the thermograms we did calculate the enthalpy variation (ΔH) associated to the binding reaction while the free energy (ΔG) was deduced from the dissociation constant (K_D) measured by dialysis. Data are shown in table I.

As it can be seen, the enthalpy variation upon binding is strongly negative when compared to the values we did obtain in a previous study of LIV-BP (13).

Also strongly negative, the values of ΔG reflect the high affinity of Gln-BP for its ligand. The entropy variation, ΔS, is very highly negative and for that reason it is the most interesting datum to be discussed below.

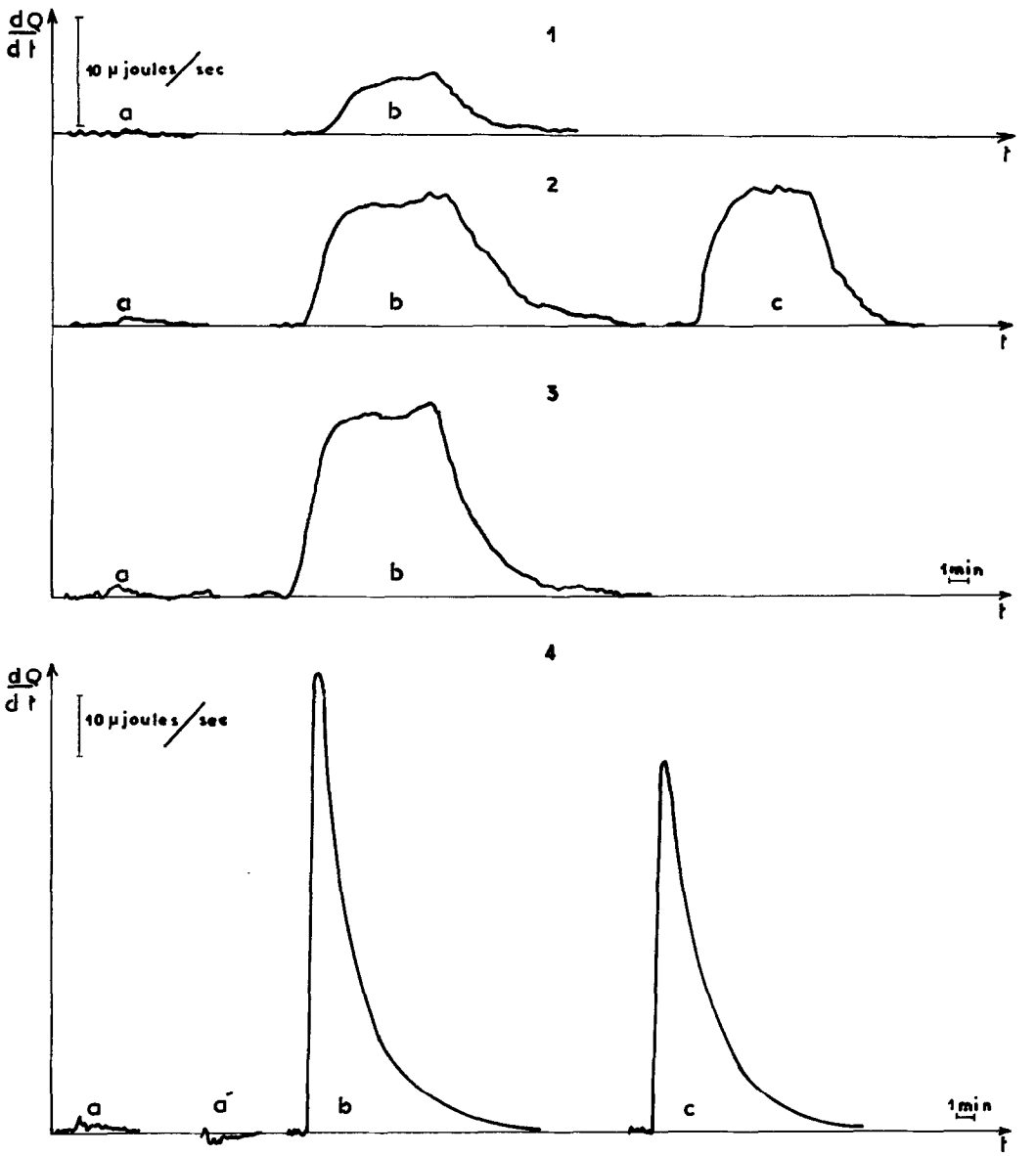

Fig. 1 - Thermograms of binding reaction.
1,2,3 : Flow calorimeter experiments (Amplifier 10 µV- Recorder 50 mV).
1 a : heat of dilution of Gln-BP (Binding sites = 0,027 µmole)
1 b : heat of reaction of Gln-BP with glutamine.
2 a and 2 b : idem but Gln-BP concentration x 2
3 a and 3 b : " " " x 4
2 c : heat of calibration corresponding to 5,06 m Joules (1,210 mcal)
4 : Batch calorimeter experiment (Amplifier 10µV - Recorder 100 mV)
a : heat of dilution of Gln-BP (Binding sites = 0,141 µmole)
a': " " Glutamine
b : heat of reaction of Gln-BP with Glutamine
c : heat of calibration corresponding to 11,21 m Joules (2,68 mcal).

Table I :
Thermodynamic parameters of glutamine fixation upon Glutamine Binding protein.

K_D µM	Binding Siters µmole	Thermogram number (cf Fig 1)	Number of experiments	Q (mean value) m cal	Q (mean value) m Joules	ΔH Kcal. mol^{-1}	ΔG Kcal. mol^{-1}	ΔS Cal. mol^{-1}. K^{-1}
0,2	0,027	1 b	2	0.571	2.39	- 21.2	- 9.13	- 40.5
"	0,054	2 b	3	1.36	5.68	- 25.4	"	- 54.6
"	0,108	3 b	2	2.305	9.63	- 21.5	"	- 41.5
"	0,141*	4 b	2	2.952	12.34	- 21	"	- 39.8

In the flow calorimeter experiments the quantity of ligand was 5 µmoles per ml and in the batch calorimeter experiments (*) the quantity of ligand in the microcalorimetric cell was 2 µmoles i.e. in both cases largely in excess when compared to the number of binding sites.

The dissociation constant (K_D) was measured according to the techniques previously published (9,15,16).

- ΔG value was deduced from the following relationship $\Delta G = -RT \ln 1/K_D$
- ΔS value was calculated according to the equation : $\Delta S = \frac{\Delta H - \Delta G}{T}$

Experimental temperature was 25 ± 0,1°C.

DISCUSSION

The three parameters (ΔG, ΔH, ΔS) that allow a full thermodynamic description of the binding reaction have been determined. From the values obtained it can be deduced that the complex formation is enthalpy driven. This is demonstrated by the results obtained from the flow and the batch calorimeters in spite of the lesser sensivity of the later.

In a study upon LIVBP (13) we found ΔH and ΔS values completely distinct from those obtained here ; we concluded that the reaction was entropy driven and that the protein, upon binding was undergoing a disorganization of water molecules near the binding site.

In the present case the highly negative values of ΔS obtained suggest that binding reaction of Glutamine to Gln-BP is accompanied by an important conformational change that may concern the entire molecule an not only the binding site.

Wiener and Heppel (9) found in their fluorescence study of Gln-BP that addition of glutamine changed the environment of the two tryptophan residues of the molecule. At that time they could not conclude whether the tryptochans were at the active site and hidden by glutamine or whether glutamine caused a conformational change burying the tryptophan residues. Results found in this study support the second hypothesis if we assume that in the first hypothesis the resulting conformational change would be probably weak since limited to the binding site.

Kreishman et al (10) too, concluded their PMR study of Gln-BP indicating that the protein was undergoing a large conformational change upon binding and that the tryptophans were buried at the same time .

On the other hand, highly negative values of ΔS allow us to say that, after binding, the protein ligand complex, including water molecules that would be associated, is in a more ordered state than the free protein in solution. Shrake et al (20) studying glutamine synthetase (GS) of E.coli found a

very similar result; their conclusion also was that the complex GS-glutamine is in a more ordered state than the protein alone.

Conformational change and very negative variation of ΔS lead us to suppose that upon binding the complete change occuring may result into formation of a Gln-BP-glutamine complex with some hydrophobic residues oriented outside and water molecules reorganized all around.

Moreover it can be pointed out that such large or very large negative ΔS variations were observed by different authors : Schleif (21) who studied the Arabinose binding protein of E.coli, Kuriki et al (22) with the (Na^+, K^+) ATPase of the electric eel and by Belaich et al (23) in their study of Thiodigalactoside and lactose binding upon βgalactoside permease Mprotein of E.coli. All these exemples, along with the present work show that enthalpic and entropic contributions are large, in absolute, but opposite when considered from energetic point of view. In these cases the enthalpic variation made the binding reaction favorable and so temperature dependent. We should like to lay emphasis on the fact that these kinds of biochemical reactions are generally thought as temperature independent and that this last concept must now be reconsidered.

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