MICROCALORIMETRIC STUDY OF SUBSTRATE FIXATION ON THE LEUCINE-ISOLEUCINE-VALINE-BINDING PROTEIN FROM ESCHERICHIA COLI

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SUMMARY

The thermodynamical behaviour of the Leucine-Isoleucine-Valine binding protein implicated in branched chain amino acids transport has been examined. The enthalpy changes (ΔH) were measured by microcalorimetry for the binding reaction of the protein with leucine, isoleucine and valine. The binding of the amino acids resulted in a slightly endothermic reaction for leucine and in exothermic reaction for both isoleucine and valine. The free energy values (ΔG) were calculated from the respective dissociation constants measured with the three substrates by the rapid dialysis technique of Colowick and Womack. From ΔG and ΔH contributions the entropic values (ΔS) corresponding to the complex formation reactions were deduced and were found highly positive in all cases.

INTRODUCTION

One of the remaining problems about the periplasmic binding proteins involved in transport is the means by which they are catalysing transfer of their substrate through the membrane (1, 2). For most authors a substrate-induced conformational change of the binding protein is necessary to permit the recognition of the protein-substrate complex by an eventual membrane receptor.

Evidences for a conformational change of the protein structure after fixation of its substrate were presented for a number of binding proteins using fluorescence, gel electrophoresis or NMR techniques (3, 9).

Another approach is described in this paper. It consists in the direct study of the binding reaction by microcalorimetric technique. Such a study including, in addition, measurements of affinity binding constants permits the thermodynamical description of the reaction which can give valuable

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informations on eventual conformational changes of the protein and (or) of the substrate.

This study was performed using Leucine-Isoleucine-Valine binding protein (LIVBP) previously purified (10, 11) and for which conformational change after binding of the substrate was observed at this time by conventional techniques (11, 13).

MATERIALS AND METHODS

Escherichia coli K12 strain 3102 gal K^- (gift from Dr. Puig) was grown in minimal medium supplemented with glucose. The LIVBP was extracted and purified to homogeneity as described previously (10, 11). The protein was discharged from eventual retained substrate by denaturation in urea 8 M then renaturation by dialysis against buffer according to Amanuma et al. (14). The determination of the protein concentrations were made using the Lowry's procedure (15). The binding for substrates was measured by the rapid dialysis technique of Colowick and Womack (16). Chemicals were analytical grade and were purchased from the Radiochemical Centre for tritiated amino acids and from Sigma, Merck or Prolabo for others chemicals. All the experiments were performed at 25° C in 0.1 M sodium phosphate buffer pH 7.0 with in addition 0.1 M sodium butyrate to prevent amino acid adsorption upon the dialysis or microcalorimetric cell walls. Microcalorimetric measurements were made in LKB microcalorimeter 2107 (LKB Producter, Sweden) by the previously published method (17) with slight modifications. For the heat of reaction measurements (Q_1) 4 ml of LIVBP preparation and 2 ml of substrate solution (concentrations are given in Table I) were introduced in the larger and the smaller compartments of the calorimetric experimental cell respectively. To evaluate the heat of dilution (Q_2) of substrates the experimental cell was charged in the same manner but replacing 4 ml of LIVBP solution by 4 ml of buffer. The heat of dilution of LIVBP was also determined and could be neglected. In all cases the reference calorimetric cell was filled with 4 ml and 2 ml of buffer. The true heat of reaction was : $Q = Q_1 - Q_2$.

RESULTS

Figure 1 shows examples of thermograms obtained for the isoleucine valine and leucine binding on the LIVBP. It appears that the binding of isoleucine and valine is an exothermic process whereas the binding of leucine is endothermic. From these thermograms the enthalpy variations (ΔH) accompagnying the binding reactions were calculated. From dissociation constants (K_D) measured by dialysis the Gibbs free energy variations (ΔG) were estimated. The entropy variations (ΔS) were deduced from ΔH and ΔG values. All the values obtained are shown on Table I.

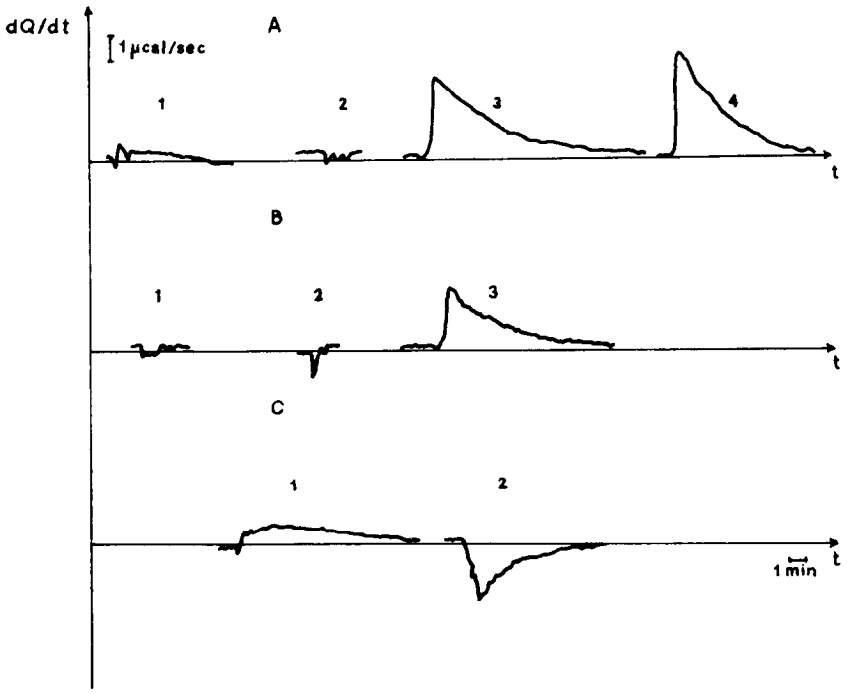

Fig. 1 - Thermograms of binding reactions.
A, 1, heat of dilution of the LIVBP. 2, heat of dilution of isoleucine. 3, heat of reaction of isoleucine with the LIVBP. 4, heat of calibration corresponding to 0.764 mcal.
B, 1, Artefact of mixing corresponding to the rotation of the calorimeter. 2, heat of dilution of valine. 3, heat of reaction of valine with the LIVBP.
C, 1, heat of dilution of leucine. 2, heat of reaction of leucine with the LIVBP.
All concentrations are indicated in Table I.

Reflecting the high affinity of the LIVBP for the three substrates, strongly negative values of ΔG were calculated.

Surprisingly the ΔH are slightly negative for the binding of isoleucine and valine when it is weakly positive for the binding of leucine. On other hand, the ΔS values are strongly positive for the three substrates binding with the higher value for the binding of leucine.

DISCUSSION

The thermodynamic function variations associated to the complexes for-

TABLE I - THERMODYNAMIC PARAMETERS OF REACTION OF BINDING OF BRANCHED CHAIN AMINO ACIDS TO THE LIV BINDING PROTEIN

In all experiments the quantity of ligand in the microcalorimetric cell was 2 µmoles i.e. more than 10 times the number of binding sites. The heat of binding (Q) is the difference between the heat of reaction and the heat of dilution of substrate. Values given are the average of two measurements. The dissociation constants (K_D) were measured by the means of the rapid dialysis technique of Colowick (16). The ΔG values were calculated from K_D according to the relationship $\Delta G = - RT \log 1/K_D$. ΔS was deduced from ΔG and ΔH values by the means of the equation : $\Delta S = \frac{\Delta H - \Delta G}{T}$. The experimental temperature was 25° C.

Ligand	K_D µM	Binding sites µmole	Q mcal	ΔH Kcal mol^{-1}	ΔG Kcal mol^{-1}	ΔS cal mol^{-1} K^{-1}
L isoleucine	0.25	0.1845	- 0.660	- 3.6	- 9.0	18.2
L leucine	0.40	0.1805	+ 0.412	+ 2.3	- 8.7	36.9
L valine	0.90	0.1845	- 0.385	- 2.1	- 8.2	20.6

mation of LIVBP with its substrates have been examined. It appears that these complexes are entropy rather than enthalpy driven.

Berman and Boyer (13) found a ΔH value in the range of - 5 kcal/mole for the binding of leucine to the LIVBP using the Van t'Hoof's relationship. This result, if quantitatively different of ours, is not in fundamental contradiction with this study because positivity of ΔS is not changed by such a ΔH. In our laboratory, recent investigations have shown discrepancies and sometime contradictions between Van t'Hoff ΔH measurements and microcalorimetric determination (18). Recently the thermodynamic analysis of the binding of isoleucine and various analogs on the L-isoleucine t RNA ligase of E. coli was done (19) and good accordance, for the binding of isoleucine, with our results can be noted. It seems of great importance that the branched chain amino acids molecules are constituted by an hydrophilic part (amin-carboxylic part) and by an hydrophobic part (aliphatic branched chain). It is known that the two parts of the amino acid are involved in the binding (11, 20). Parti-

cularly, the amino group and the α-hydrogen atom in the hydrophilic part and the β and γ hydrogen atoms in the hydrophobic part seem to be necessary for the specificity of the reaction. It was assumed by Amanuma and Anraku (20) that the β hydrogen atom was in the best configuration in the case of isoleucine i.e. when the β carbon rotation is reduced by the branched methyl.

Recently we have pointed out by NMR studies that the methyl groups of the aliphatic chain and the α proton of isoleucine are all perturbated by the binding reaction with the largest effect on the α proton (21).

All these observations support the idea that a coulombic interaction with a negative enthalpy change would occur between the protein and the charged part of the amino acid molecule. However the thermodynamics of the binding reaction described here show that this reaction is characterised by a large increase in entropy with enthalpy variation near zero. Such a phenomenon is favorable to a predominance of hydrophobic interactions between the protein and its substrates. Particularly the large ΔS values would be compatible with the elimination of the organized water molecules, so called "iceberg" (22), around the hydrophobic parts of the amino acids during the reaction.

So the possible effect of the coulombic interaction described above would be thermodynamicaly hidden by the endothermic melting of the "icebergs" which occurs during hydrophobic interactions. This assumption is in good agreement with the fact that the leucine molecule which presents the most hydrophobic character is the only one which has a positive enthalpic effect during the complex formation reaction.

In conclusion, we can assume, as other authors (11, 20), that the two parts of branched chain amino-acids are involved in the binding with the LIVBP. However the variability in affinity seems to be in relation with the aliphatic chain by two characteristics which are the reduction of β-carbon rotation and the lenght of the chain explaining that affinity of the LIVBP for isoleucine is higher than that for leucine. Concerning the conformation change of the protein, the binding seems accompanied by a great disorder in the

"icebergs" organized water molecules. As no important change was detected by various techniques (11, 13) it can be assumed that the disorganisation of water molecules occurs only near the binding site of the protein.

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