ESCHERICHIA COLI LACTOSE REPRESSOR

Isolation of two different homogeneous headpieces and the existence of a hinge region between residues 50 and 60 in the repressor molecule

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1. Introduction

The lactose repressor controls the expression of the *lac* genes in *E. coli*. The specific binding of the repressor to the *lac* operator DNA segment inhibits the transcription of the polycistronic messenger RNA representing the structural genes of the *lac* operon (reviewed [1,2]). *Lac* repressor shows not only the base-specific strong binding to operator DNA but also a much weaker interaction with DNA containing non-operator base sequences [3]. There is overwhelming genetic and biochemical evidence indicating that the amino-terminal segments ('headpieces') of the tetrameric *lac* repressor are a necessary requirement for both operator and non-operator DNA binding [3–6]. We have recently shown that selective trypsin cleavage of native *lac* repressor under highly restricted conditions (1 M Tris–HCl, 30% in glycerol, pH 7.5, room temp.) produces a mixture of monomeric 'headpieces' accounting for residues 1–51 and 1–59 together with a trypsin resistant tetrameric core [7]. The tetrameric repressor core is devoid of all detectable DNA binding activities [3,5], whereas the headpieces retain the ability to form complexes with non-operator DNA but do not show the high specificity of repressor for operator sequences [8]. The two headpieces can be separated on DNA-cellulose and show different DNA binding properties [8]. In order to facilitate further studies on the interaction of headpieces with DNA we have probed the repressor structure under native conditions with two other proteolytic enzymes in the hope of obtaining homogeneous headpieces. Here we show that chymotrypsin gives rise to a single homogeneous headpiece covering amino acid residues 1–56, and that clostripain gives rise to a headpiece covering amino acid residues 1–51.

2. Materials and methods

Purification of *E. coli* repressor and its storage has been described [5]. Digestions were performed in 1 M Tris–HCl (pH 7.5), 30% in glycerol at room temp. using 10 mg/ml repressor protein and 2% (w/w) either chymotrypsin (Serva, Heidelberg) or clostripain (Worthington, Freehold). After 2 h, digestion was stopped by addition of a 5-fold weight excess of turkey egg white trypsin inhibitor Type II-T (Sigma, St Louis) to bind the chymotrypsin or by addition of ethylene-glycol-bis (β-amino ethyl ether)-*N,N'*-tetra acetic acid (EGTA) in 1 M Tris–HCl, pH 7.5, to final conc. 2×10^{-2} M to inhibit clostripain. Separation and purification of headpiece peptides using chromatography on Sephadex G-150 and G-25 has been described [7]. Columns were equilibrated with 0.1 M NH_4HCO_3, 10^{-3} M in NaN_3. In case of the chymotryptic headpiece phenylmethylsulfonyl-fluoride was added at 2×10^{-4} M. In case of the clostripain headpiece EGTA was present at 10^{-2} M. Purity of the preparations was checked by electrophoresis in sodium dodecylsulfate containing 20% polyacrylamide gels and by standard amino acid analysis as in [7]. Digestion with carboxypeptidases A and B was as in [7]. The amino-terminal residues were determined by the dansyl-procedure [9].

3. Results

The headpieces resulting from digestion of repressor in 1 M Tris–HCl, pH 7.5, by clostripain or chymotrypsin were purified by chromatography on Sephadex G-150 and G-25 (see section 2) as described for the headpieces obtained by trypsin [7]. Purity and homogeneity of the resulting headpieces have been checked by several criteria. Both headpieces electrophorese on 20% polyacrylamide gels containing dodecylsulfate as single polypeptides (fig.1). Amino acid analysis of both preparations (table 1) indicates homogeneity assuming that the clostripain and chymotryptic headpieces cover amino acid residues 1–51 and 1–56, respectively (fig.2). Although this assumption is in agreement with the known specificity of the enzymes used, digestion

Table 1
Protein chemical data of different headpieces

1. Amino acid composition

	Chymotryptic headpiece		Clostripain headpiece	
	Found	Theoretical	Found	Theoretical
Asp	3.68	4	4.31	4
Thr	2.78	3	2.28	3
Ser	3.89	4	3.94	4
Glu	7.55	8	6.00	6
Pro	2.20	2	2.09	2
Gly	1.27	1	1.75	1
Ala	7.39	8	6.30	7
Val	7.50	9	6.36	8
Met	1.79	2	1.48	2
Ile	1.31	1	1.19	1
Leu	2.81	3	2.32	2
Tyr	3.56	4	3.21	4
Lys	3.21	3	3.04	3
His	0.99	1	0.96	1
Arg	2.79	3	2.50	3

2. Amino acids released by carboxypeptidases

	Amino acids	Released (nmol)	Amino acids	Released (nmol)
	Leu	2.6	Arg	6.9
	Gln	5.5		
	Ala	2.6		
	Val	2.4		
Total peptide digested		2.5		6.9

3. Amino-terminal residues: Met (chymotryptic headpiece); Met (clostripain headpiece)

(1.) Amino acid composition of chymotryptic and clostripain headpieces. Theoretical values are taken from the amino acid sequence [10]. (2.) Amino acids released from the chymotryptic headpiece by complete digestion with carboxypeptidase A and from the clostripain headpiece by complete digestion with carboxypeptidase B. Amino acids released from the chymotryptic headpiece appear in the order given (see also fig.2). (3.) The amino-terminal residue of both headpieces was determined by the dansyl-method [9]

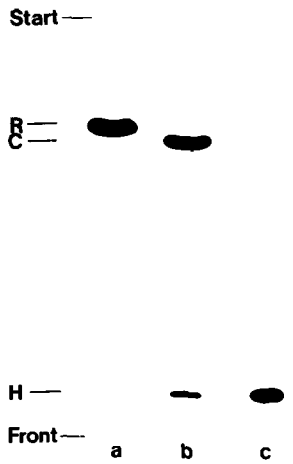

Fig.1. Dodecylsulfate–polyacrylamide gel electrophoresis of repressor before (a) and after (b) digestion with chymotrypsin in 1 M Tris–HCl (pH 7.5), 30% in glycerol, and of the purified chymotryptic headpiece (c). R, C and H indicate repressor, core and headpiece.

with carboxypeptidases B and A was used to further verify this assignment (table 1). In addition both headpieces have methionine as amino-terminal residue. This residue corresponds to residue 1 of the *lac* repressor polypeptide chain [5,10].

4. Discussion

Native *lac* repressor can be digested by a variety of proteolytic enzymes in ammonium bicarbonate buffer to yield a protease resistant tetrameric core

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 1                5                10               15
Met-Lys-Pro-Val-Thr-Leu-Tyr-Asp-Val-Ala-Glu-Tyr-Ala-Gly-Val-
16               20               25               30
Ser-Tyr-Gln-Thr-Val-Ser-Arg-Val-Val-Asn-Gln-Ala-Ser-His-Val-
31               35               40               45
Ser-Ala-Lys-Thr-Arg-Glu-Lys-Val-Glu-Ala-Ala-Met-Ala-Glu-Leu-
46               50  a▼b          55  c▼       b▼60
Asn-Tyr-Ile-Pro-Asn-Arg-Val-Ala-Gln-Gln-Leu-Ala-Gly-Lys-Gln-
61               65
Ser-Leu-Leu-Ile-Gly-------►
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Fig.2. Amino acid sequence of the amino-terminal 65 residue of *lac* repressor [10]. Numbers above the residues give the residue number. The arrows mark the points of cleavage in 1 M Tris–HCl (pH 7.5), 30% in glycerol by clostripain (a), trypsin (b) and chymotrypsin (c).

missing up to 59 of the amino-terminal residues, which are released in form of smaller peptides ([5,7,11], Beyreuther cited [1]). When digestion with trypsin was performed in the presence of 1 M Tris–HCl, pH 7.5, enzymatic cleavage was severely restricted and only the peptide bonds of arginine 51 and lysine 59 were opened [7]. Thus the isolation of a mixture of two amino-terminal headpieces (residues 1–51 and residues 1–59) became possible [7]. Here we have shown that the same buffer also severely restricts the proteolytic action of two other enzymes, clostripain and chymotrypsin. Therefore homogeneous headpieces accounting either for residues 1–51 or for residues 1–56 can be isolated. These results indicate that the *lac* repressor molecule must have a very protease resistant structure in 1 M Tris–HCl, which with the enzymes used only allows proteolytic attacks between amino acid residues 50 and 60. This region therefore must have a weak secondary structure and probably forms a hinge region. It is interesting to note that genetic analysis has shown that some strong DNA binding mutants are localized within this sequence [1,6].

We have shown previously that the isolated tryptic headpieces (residues 1–51 and 1–59) show the same differential trypsin sensitivity as isolated peptides as they show when they are part of the tetrameric *lac* repressor. They are resistant to trypsin in 1 M Tris–HCl buffer but readily susceptible to tryptic digestion in 0.05 M Tris–HCl buffer [8]. Thus the use of high molarity Tris buffer must have a pronounced effect on the structure of *lac* repressor. This effect is not found with phosphate buffers or neutral salts like NaCl, which do not stabilize the headpieces. Thus we expect that Tris must specifically stabilize a special conformation of *lac* repressor and its headpieces. This assumption is in line with the relatively high solubility of repressor in 1 M Tris–HCl buffer when compared with other buffers of similar ionic strength. It remains to be seen whether this action of Tris reflects an interaction of repressor in vivo with a currently unknown component, like an amine, or if it is purely fortuitous.

The chymotryptic headpiece (residues 1–56) should be very useful for further physical–chemical characterization of protein–nucleic acid interaction, since it is readily available in a homogeneous form and is only three amino acid residues shorter than

the longer tryptic headpiece. The latter peptide however has to be separated by a further chromatography step from the contaminating shorter tryptic headpiece.

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