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Isolation of the Amino-Terminal Fragment of Lactose Repressor Necessary for DNA Binding†

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ABSTRACT: *lac* repressor can be dissected by trypsin into a homogeneous tetrameric core (accounting for residues 60 to 347), carrying inducer binding activity, and the monomeric amino-terminal peptides ("headpieces") accounting for residues 1 to 59 and 1 to 51, respectively. This restriction of the action of trypsin on *lac* repressor is obtained in 1 M Tris-HCl (pH 7.5)–30% in glycerol at 25 °C since only the peptide bonds at lysine-59 and to a lesser extent at arginine-51 are cleaved under these conditions. The headpieces can be purified by gel filtration. They have ordered secondary structure as revealed by circular dichroism studies. The monomeric headpieces show the relatively weak binding to nonoperator DNA but not the highly specific and strong binding to operator DNA typical for tetrameric *lac* repressor.

The lactose repressor is probably the best characterized protein regulating gene expression in *E. coli*. Specific binding of repressor to *lac* operator DNA controls the lactose genes. Binding of an inducer such as isopropyl β-D-thiogalactoside to repressor reduces the affinity to the operator greatly and allows expression of the lactose genes (for a review, see Müller-Hill, 1975).

There have been two main approaches—genetics and biochemistry—to locate the region(s) within the *lac* repressor polypeptide chain which are responsible for DNA binding. Genetic analysis has revealed that the majority of the mutations which lead to a defective operator binding site but do not disturb either the binding of inducer or the tetrameric structure of repressor are located within the amino-terminal 60 residues of the repressor polypeptide chain (Adler et al., 1972). The same result has been obtained by an extensive study on the suppression of nonsense mutants in the *lac* i gene (Miller et al., 1975). The biochemical approach has used the fact that the action of trypsin on *lac* repressor is very restricted (Platt et al., 1973). Thus it was possible to isolate a homogeneous tryptic core which has lost the amino-terminal 59 residues of the polypeptide chain. This core had full inducer binding activity and tetrameric structure but was inactive in binding to nonoperator or to operator DNA (Platt et al., 1973; Files and Weber, 1976). Repressor derivatives with similar structural and functional properties could also be isolated from some repressor mutant strains due to translational reinitiation past a nonsense codon (Platt et al., 1972; Ganem et al., 1973; Files et al., 1974). The combined genetic and biochemical evidence makes it very likely that the amino-terminal region of the *lac* repressor is directly involved in the strong and specific binding to *lac* operator DNA as well as the weak binding to nonoperator DNA (Lin and Riggs, 1975). Thus far, however, it has not been possible to isolate the amino-terminal part of the repressor and to see if it retains DNA binding properties related to those of the intact repressor.

Here we report the conditions for limited tryptic digestion which allow the dissection of native repressor into a homogeneous tetrameric core and monomeric amino-terminal headpieces. The headpieces have ordered secondary structure and show the weak binding to nonoperator DNA typical for *lac* repressor (Lin and Riggs, 1972, 1974; von Hippel et al., 1974) but not the specific and strong binding to operator DNA.

Materials and Methods

Tryptic Digestion of lac Repressor under Native Conditions. Two main digestion conditions were used: (a) digestion at 25

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°C in 0.1 M NH_4HCO_3 as previously described in detail (Files and Weber, 1976); (b) digestion at 25 °C in repressor *storage buffer* (1 M Tris-HCl (pH 7.5), 30% in glycerol, and 0.01 M in ME[1]). The latter digestion conditions, which for the first time permit the isolation of the repressor headpieces, are as follows: One volume of *lac* repressor at 6 mg/mL in storage buffer is brought to room temperature and trypsin (Worthington) dissolved in storage buffer is added at a concentration equal to 1% by weight of the repressor. The reaction is stopped after 2 h by the addition of soybean trypsin inhibitor (Worthington) at a fivefold excess by weight over the amount of trypsin present in the solution.

Isolation of the Headpieces. Repressor was digested for 2 h at 25 °C in storage buffer under native conditions as described above. Fifteen minutes after the addition of trypsin inhibitor, the solution (1 mL) was applied to a Sephadex G-150 column (1.5 cm diameter; 135 cm in length) previously equilibrated with 0.1 M NH_4HCO_3. The elution profile of the column was monitored by measuring the optical density at either 280 or 230 nm. The fractions containing either the tryptic core or the headpieces were pooled. Some experiments on the headpieces were done with the pooled material. In others the material was recovered by lyophilization and then dissolved in the appropriate buffer. Purity of the peptide was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 20% acrylamide slabs (Laemmli, 1970).

DNA-Binding Assay. The binding of the headpieces to DNA was assayed by the filter binding procedure (Bourgeois, 1971). The binding buffer contained 0.01 M Tris-HCl (pH 7.4), 0.01 M KCl, 10^{-3} M EDTA, 10^{-3} M dithiothreithol, 5% dimethyl sulfoxide, 3×10^{-3} M $MgCl_2$, and 50 μg/mL bovine serum albumin. The reaction volume was 0.3 mL, of which 0.28 mL was filtered through Millipore AAWP nitrocellulose filters, 0.8 μm pore size and 25 mm in diameter. The reaction was performed at room temperature.

Circular Dichroism. Spectra were recorded on a Cary 60 spectropolarimeter with CD attachment. Path length of the cell was 0.1 cm. The mixture of headpieces was studied in 0.1 M NH_4HCO_3 as prepared by the Sephadex G-150 column. In other experiments the lyophilized headpiece material was dissolved in various buffers prior to spectral analysis. The concentration of the proteins was determined by quantitative amino acid analysis of a small aliquot of the solution.

Miscellaneous Procedures. Amino acid analysis was performed on a Biotronic Amino acid analyzer using the 0.5–2-nmol range and a single column buffer system. Amino-terminal residues were characterized by the micro-dansyl-technique (Hartley, 1970). Digestion with carboxypeptidase B (Worthington) was performed at an enzyme-to-substrate ratio of 1:100 in 0.05 M Tris-HCl (pH 7.8). The carboxy-terminal residues were identified using the amino acid analyzer. Cleavage with CNBr at methionine residues was performed in 70% formic acid for 20 h. Peptides were separated on Whatman No. 1 paper using high-voltage electrophoresis (0.2 M pyridine acetate (pH 6.5)) in the first dimension and ascending chromatography in the second dimension (Waley and Watson, 1953). Peptides were detected after light spraying of the paper with 0.01% ninhydrin in 95% ethanol. The spots were cut out and the peptides eluted from the paper by 1 M ammonia and recovered by lyophilization.

[1] Abbreviations used: ME, 2-mercaptoethanol; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; WT, C, and H, wild type, homogeneous core, and headpiece polypeptide chains, respectively.

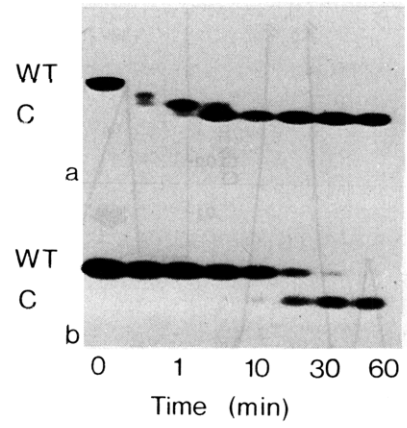

FIGURE 1: Time course of tryptic digestion of *lac* repressor under native conditions analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10% slab gel). Buffer conditions are 0.1 M NH_4HCO_3 in a and 1 M Tris-HCl (pH 7.5)–30% glycerol–0.01 M ME in b. WT and C mark the polypeptide chains of normal repressor and its homogeneous core. Note the presence of polypeptides with intermediate molecular weights early during digestion in a and the absence of these bands in b.

Results

1. Tryptic Cleavage of lac Repressor into Core and Headpieces. Limited tryptic digestion of *lac* repressor was monitored by sodium dodecyl sulfate gel electrophoresis using 10% polyacrylamide slab gels. Figure 1a shows a time course for the digestion of repressor in 0.1 M NH_4HCO_3 at 25 °C as described previously (Files and Weber, 1976). The gel pattern is characterized by a rapid conversion of the wild type polypeptide chain (WT) to the homogeneous core polypeptide chain (C). Typical of this digest is the presence of bands with molecular weights intermediate between those of WT and C. These intermediate species visible at early times during the digest (0.5 to 5 min) are indicative of the action of trypsin at the various lysine and arginine residues in the amino-terminal region of the polypeptide chain. The end products of the digest are a homogeneous tetrameric core missing only the 59 amino-terminal residues of the *lac* repressor polypeptide chain and a series of tryptic peptides accounting for the amino-terminal 59 residues (Files and Weber, 1976). Thus cleavage of the polypeptide chain resulting in digestion of the amino-terminal end is characterized by intermediate size polypeptides on a sodium dodecyl sulfate gel. Therefore we tried a variety of digestion conditions in the hope of detecting by the absence of intermediate size polypeptide chains those conditions which give rise to the full size amino-terminal 59 residues.

When repressor is digested at 25 °C in storage buffer (1 M Tris (pH 7.5), 30 or 50% in glycerol, and 0.01 M in ME) the action of trypsin is slowed down very strongly (Figure 1b). After 5 to 10 min a slow conversion of the WT polypeptide to the C polypeptide becomes noticeable. Completion of the cleavage requires between 1 and 2 h. The time course experiment also shows that the intermediate size polypeptides typical of the digestion in 0.1 M NH_4HCO_3 are conspicuously absent. We show below that digestion in storage buffer leads to intact headpieces and a homogeneous core.

When repressor, dialyzed against storage buffer in order to remove residual ammonium sulfate ions present from the last purification step (Platt et al., 1973), is digested as above, the same pattern as in Figure 1b is obtained. Thus ammonium sulfate is not responsible for the difference in the digestion patterns seen in Figures 1a and 1b. The following buffer systems (1) 0.05 M Tris-HCl (pH 7.5)–30% in glycerol, (2) 0.1

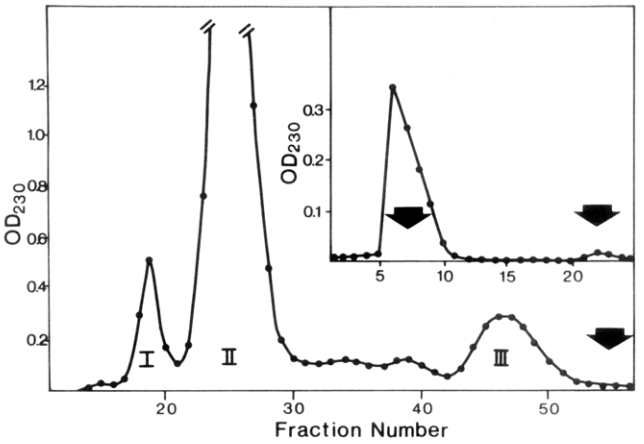

FIGURE 2: Purification of the repressor headpiece by chromatography on Sephadex G-150. The recovered headpiece (peak III) was further chromatographed on Sephadex G-25 (insert), where it appears in the void volume or on Sephadex G-100 (not shown). Peak II from the G-150 column is the tryptic core. The arrows mark the salt volume of the columns.

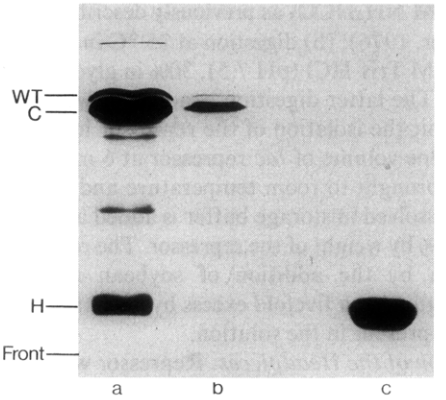

FIGURE 3: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 20% slab gel. (a) Repressor in 1 M Tris-HCl–30% glycerol digested for 60 min; (b) peak II from the Sephadex G-150 column; (c) the headpiece preparation from the Sephadex G-150 column. Slots a and c are heavily overloaded in order to demonstrate the purity of the peptide. WT, C, and H mark the positions of repressor polypeptide, core polypeptide, and headpiece polypeptide, respectively. Note that the repressor in this experiment has not been quantitatively converted to core.

M NH_4HCO_3–30% in glycerol gave digestion patterns similar to those found in 0.1 M NH_4HCO_3 shown in Figure 1a. Thus the presence of a high concentration of Tris-HCl is necessary to restrict the action of trypsin and to recover the headpieces (see below).

2. Isolation of the Headpieces. lac repressor was digested at 25 °C in storage buffer for 2 h as described above. The reaction was stopped by the addition of soybean trypsin inhibitor and the digest applied on a Sephadex G-150 column in 0.1 M NH_4HCO_3 at room temperature. Figure 2 shows the elution profile obtained from the column. The three peaks were characterized by sodium dodecyl sulfate gel electrophoresis on 20% polyacrylamide slabs. Peak I contains a small amount of aggregated repressor which we have not characterized further. Peak II contains the homogeneous tryptic core, slightly contaminated by some undigested repressor. Peak III contains the headpieces. Sometimes the preparation of headpieces was recovered by lyophilization, redissolved in 0.1 M NH_4HCO_3, and subjected to gel filtration on Sephadex G-25 (Figure 2, insert) or Sephadex G-100 (data not shown). Figure 3 shows a sodium dodecyl sulfate slab gel which has been heavily overloaded to show the purity of the fractions containing the headpiece. Slot a contains the unfractionated digest, slot b an aliquot of the fraction containing the core, and slot c the isolated headpieces. When the headpieces purified on two Sephadex columns were loaded on the gel at 60 μg, no bands corresponding either to repressor (WT) or core (C) could be detected. The molecular weight of the headpieces under native conditions was estimated on the calibrated Sephadex G-100 column and was found to be approximately 6000.

3. Protein Chemical Characterization of the Headpieces and Core. The headpieces isolated by Sephadex G-150 gel chromatography were further purified on Sephadex G-25 in order to assure their separation from a small tryptic peptide of eight amino acids which might be present (see below). Table I shows the amino acid composition of the repressor headpieces as well as the determination of the amino-terminal and carboxy-terminal residues. Although there is only one amino terminal residue (methionine), carboxypeptidase B digestion reveals the presence of both lysine and arginine in nearly equal molar amounts. The amino acid composition and the end group analyses together with the known amino acid sequence of the repressor (Beyreuther et al., 1973) make it very likely that the

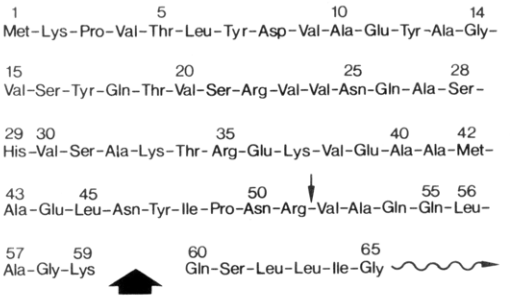

FIGURE 4: The amino acid sequence of the amino-terminal 65 residues of *lac* repressor (Beyreuther et al., 1973). Numbers above the residues give the residue number. The two arrows mark the points of cleavage by trypsin in 1 M Tris-HCl (pH 7.5)–30% in glycerol. The thick arrow marks the nearly quantitative cleavage at residue 59; the thin arrow marks the incomplete cleavage site at position 51. For protein chemical characterization, the headpiece preparation (nearly equal amounts of peptides 1–59 and 1–51) was cleaved with CNBr at methionine residues 1 and 42, and the resulting soluble peptides BrCN1 (residues 43 to 51) and BrCN2 (residues 43 to 59) were recovered by fingerprinting (see Table I). The tryptic peptide T6 spans amino acid residues 52 to 59.

headpiece fraction contains two peptides in nearly equal amounts. One peptide spans residues 1 to 51 and one peptide spans residues 1 to 59 of the repressor polypeptide. Table I shows that the amino acid composition of the headpiece mixture is in good agreement with this assumption. In order to verify this assignment, the headpiece peptides were subjected to CNBr cleavage. In agreement with the known amino acid sequence of *lac* repressor (Beyreuther et al., 1973; see also Figure 4) dansylation revealed two new amino-terminal residues, lysine and alanine, thus confirming cleavage at methionine residues 1 and 42. The soluble peptides of the CNBr digest were separated by two-dimensional fingerprints. Two peptides (CNBr1 and CNBr2) were found and characterized by amino acid composition and end group analysis. In agreement with the known amino acid sequence of *lac* repressor (see Figure 4), CNBr1 (neutral peptide) accounts for residues 43 to 51 and CNBr2 (basic peptide) for residues 43 to 59 (see Table I). Thus the headpiece mixture contains nearly equal parts of two polypeptides, one spanning residues 1 to 51 and the other spanning residues 1 to 59 of the *lac* repressor polypeptide chain. So far we have not been able to separate the two peptides under

TABLE I: Protein Chemical Data of the Headpiece.

	Headpiece					
	Theor[a]			CNBr 1[c]	CNBr 2[c]	T_6[c]
	1-51 (A)	1-59 (B)	Found[b]	43-51	43-59	52-59
			1. Amino Acid Composition			
Asp	4	4	4.30 (4.0)	2.10 (2)	2.11 (2)	
Thr	3	3	3.10 (3.0)			
Ser	4	4	3.63 (4.0)			
Glu	6	8	7.22 (7.0)	1.03 (1)	3.08 (3)	2.07 (2)
Pro	2	2	2.11 (2.0)	0.93 (1)	0.91 (1)	
Gly	1	2	1.83 (1.5)		1.10 (1)	0.94 (1)
Ala	7	9	7.19 (8.0)	0.91 (1)	2.60 (3)	2.00 (2)
Val	8	9	7.93 (8.5)		1.01 (1)	1.12 (1)
Met	2	2	1.84 (2.0)			
Ile	1	1	1.04 (1.0)	0.98 (1)	0.96 (1)	
Leu	2	3	2.65 (2.5)	0.99 (1)	1.94 (2)	0.95 (1)
Tyr	4	4	3.66 (4.0)	0.74 (1)	0.79 (1)	
Lys	3	4	3.45 (3.5)		0.97 (1)	0.96 (1)
His	1	1	0.95 (1.0)			
Arg	3	3	2.82 (3.0)	0.88 (1)	0.89 (1)	
			2. Terminal Amino Acid Residues			
Amino-terminal end group			Met	Ala	Ala	Val
Carboxy-terminal end group			Lys + Arg	Arg	Lys	Lys

[a] Calculated from the amino acid sequence (Beyreuther et al., 1973). [b] Amino acid composition of the headpiece preparation. The theoretical values in parentheses are calculated under the assumption that both species A and B were present in equal amounts. [c] Values in parentheses are taken from the amino acid sequence (Beyreuther et al., 1973).

conditions where they retain their biological activity (see below).

The tryptic core resulting in the native digest of repressor was subjected to amino-terminal analysis. Only one amino-terminal residue, glutamic acid/glutamine, was found. This core preparation was also compared in high-resolution sodium dodecyl sulfate–polyacrylamide gel electrophoresis with tryptic core prepared by digestion in 0.1 M NH_4HCO_3. The latter core preparation has been shown previously to lack the first 59 amino acid residues of the *lac* repressor polypeptide chain and to have an intact carboxy-terminal region (Files and Weber, 1976). The two core preparations are indistinguishable by gel electrophoresis and amino-terminal analysis.

The protein chemical characterization of the repressor headpieces and the homogeneous tryptic core predicts that approximately half of the repressor subunits in the native digest must give rise to the short octapeptide spanning residues 52 to 59. In order to challenge this interpretation, we separated the digest from the salts and glycerol by gel filtration on Sephadex G-10 in 0.1 M NH_4HCO_3. The broad peak in the void volume was pooled. The peptide material was recovered by lyophilization and extracted with electrophoresis buffer. The insoluble core was removed by centrifugation and the soluble material was subjected to two-dimensional-fingerprint separation. Two peptides were found. One peptide had hardly moved from the origin and had an amino acid composition identical with that of the headpiece mixture. The other peptide had a position in the map identical with that of the tryptic peptide T_6 of *lac* repressor (Beyreuther et al., 1975). This peptide was shown by amino acid composition and amino-terminal and carboxy-terminal end-group analysis (Table I) to be identical with peptide T_6 which spans amino acid residues 52 to 59 of the *lac* repressor polypeptide chain. This finding supports the conclusion drawn from the characterization of the repressor headpiece mixture (see above). Thus digestion of repressor in storage buffer (1 M Tris (pH 7.5)–30% in glycerol–0.01 M in ME) gives rise to homogeneous tetrameric tryptic core (residues 60 to 347), an unresolved mixture of monomeric repressor headpieces in nearly equal amount (residues 1 to 51 and 1 to 59) and the repressor octapeptide accounting for residues 52 to 59. We have not been able to change the nearly equal molar distribution between the two peptides (1-51 and 1-59) appreciably by addition of either inducer (isopropyl β-D-thiogalactoside) or antiinducer (*o*-nitrophenyl fucoside) at 0.01 M concentration during the digestion.

4. Nonoperator DNA Binding Properties of the Headpieces. The binding of the headpieces to nonoperator DNA was assayed with radioactively labeled DNA using the nitrocellulose filter binding assay described by Bourgeois (1971). Three different DNAs were used and the results are summarized in Table II. At saturating conditions, 20 μg/mL of headpieces (approximately 3×10^{-6} M) retains 80 to 88% of the input DNA (0.5 to 1.0 μM concentration of nucleotides) on the filter. In the absence of the headpieces, only a marginal background binding of the DNA to the filter can be found (1-3%). Specific and tight binding of *lac* repressor to operator DNA is found at a much lower protein concentration (approximately 10^{-12} M) (Gilbert and Müller-Hill, 1967; Riggs et al., 1968). Since the headpieces do not bind to the operator containing λ plac DNA at a similarly low concentration, the binding detected for all three DNAs at the higher headpiece concentration (10^{-6} M) reflects the weak and not sequence-specific nonoperator DNA binding typical of *lac* repressor in this concentration range (Lin and Riggs, 1972, 1974; von Hippel et al., 1974).

The circular dichroism spectrum of the headpiece preparation in 0.1 M NH_4HCO_3 is shown in Figure 5. This preparation was taken directly from the Sephadex G-150 gel-filtration step. Spectra of the mixture of headpieces taken in buffers other than 0.1 M NH_4HCO_3 (data not shown) differ slightly from that shown in Figure 5.

TABLE II: DNA Binding Data.

DNA	Concn: Nucleotides (μM)	Concn: Headpieces (μM)	cpm: Bound	cpm: Background	cpm: Total	% Retention
1. Binding of Headpieces to Different DNAs under Saturation Conditions						
[^{3}H]poly(dAT)	0.5	3	1 311	44	1 440	88
[^{32}P]-λ^a	1.0	3	21 080	300	25 980	80
[^{32}P]-λ-plac^b	1.0	3	20 970	300	23 490	88
2. Comparison of Headpieces (HP) and Repressor (R) with Respect to Operator Binding						
^{32}P-λ plac^b	0.5	0.16 (HP)	70	80	2 500	0^c
^{32}P-λ plac^b	0.2	6×10^{-4} (R)	4 932	131	5 322	90^c

^{a} Purified from strain W8 (λ CI_{857s7}). ^{b} Purified from strain BMH 782:[lac,pro]$_\Delta$ (λ CI_{857s7}plac5i$^-$o$^+$z$^+$y$^-$a$^-$) (both strains were from B. Müller-Hill). ^{c} Binding to the operator containing λ plac DNA as well as to other DNAs cannot be detected below a headpiece concentration of 3×10^{-7} M, whereas binding of repressor to operator DNA can be detected at repressor concentrations as low as 10^{-12} M or less.

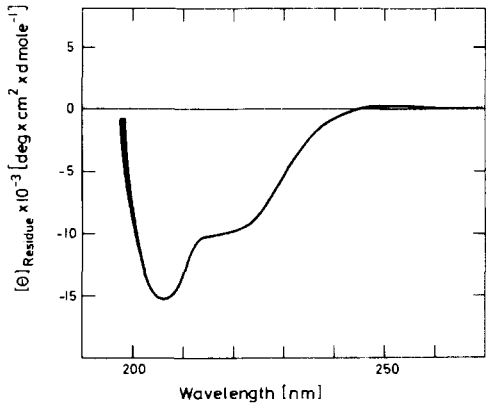

FIGURE 5: Circular dichroism spectrum of the repressor headpiece preparation in 0.1 M NH_4HCO_3. The mean residue weight was calculated from the amino acid sequence (Beyreuther et al., 1973) assuming that the preparation contained only the longer polypeptide species (residues 1 to 59; see text).

Discussion

We have shown that the action of trypsin on *lac* repressor kept at 25 °C in 1 M Tris-HCl (pH 7.5)–30% in glycerol is very restricted. After 1–2 h only the peptide bond at lysine-59 and to a lesser extent the peptide bond of arginine-51 are cleaved. The protein chemical characterization of the digestion products shows a homogeneous tetrameric tryptic core accounting for residues 60 to 347 and two monomeric repressor headpieces in nearly equal amounts accounting for the amino-terminal part of the molecule (i.e., residues 1 to 59 and 1 to 51) together with an octapeptide accounting for the balance (i.e., residues 52 to 59).

So far we do not know what is the first target of trypsin. There are several possibilities to explain our results. First, trypsin could attack approximately half of the molecules or subunits at either residue 51 or 59. The molecules or subunits which have been split at residue 51 could then in turn give rise in a second very rapid action of trypsin to the octapeptide T_6. Second, trypsin could first cut exclusively at residue 59, giving rise to a homogeneous core (residues 60 to 347). Subsequently slow hydrolysis at residue 51 could occur. Further experiments are necessary to distinguish between these two models. Such experiments are especially important since our finding of nearly equal amounts of the two headpieces could also mean that half of the molecules in the preparation or half of the subunits of the tetramers have different conformations which can be distinguished by their differential sensitivity against trypsin.

The native digestion of repressor has now been studied under a variety of different experimental conditions. Four main types of proteolytic attack can be distinguished. First, digestion in 0.1 M NH_4HCO_3 at 37 °C leads to a rapid digestion in the amino-terminal 59 residues and a slower digestion at the carboxy-terminal 40 residues. During prolonged digestion, the otherwise tetrameric core gives rise to dimer formation (Platt et al., 1973). Second, digestion in 0.1 M NH_4HCO_3 at 25 °C leads to decreased sensitivity of repressor, and it becomes possible to isolate a homogeneous tetrameric core accounting for residues 60 to 347. The amino-terminal region is digested by multiple tryptic attack and yields individual tryptic peptides (Files and Weber, 1976). Third, digestion in 1 M Tris (pH 7.5)–30% in glycerol at 25 °C restricts the access to repressor so much that only peptide bonds 51 and 59 are broken within 1 to 2 h at 25 °C. Thus for the first time the amino-terminal region can be isolated in the form of two headpieces accounting for residues 1 to 59 and 1 to 52. Four, digestion in 0.1 M NH_4HCO_3 for 2 h at 5 °C has only been characterized as far as the resulting core is concerned. The core was shown to start at either residue 52 or 60 (Huston et al., 1974). The combined results can only be explained by assuming that the tetrameric repressor has different conformations under different experimental conditions, and that these can be distinguished by their differential sensitivity against trypsin. It should be noted that repressor under native conditions is not attacked by various proteases past residue 60 (Beyreuther et al., cited in Müller-Hill, 1975; Platt et al., 1973). Since we have now shown that the region prior to arginine residue 51 can be protected against proteolytic attack by trypsin or chymotrypsin, it is likely that repressor has a "weak" secondary structure, i.e., a hinge region between residues 50 and 60. It is interesting to note that genetic analysis has shown that some strong i^{-D} mutants are localized in this hinge region (Adler et al., 1972; Miller et al., 1975; Müller-Hill, 1975).

Preliminary circular dichroism studies of the headpiece mixture show that the preparation has ordered secondary structure. This spectrum is different from those reported previously for wild type repressor and tryptic core (Chou et al., 1975; Huston et al., 1974). Since we have noticed some influence of the buffer system on the spectra, it seems premature to compare the helical content observed with the one estimated

from the amino acid sequence (Chou et al., 1975) until we have found conditions where we can compare faithfully the spectra for repressor, homogeneous core, and headpieces.

The *lac* repressor has two DNA binding activities. The first is the strong and specific binding to the *lac* operator DNA (Gilbert and Müller-Hill, 1967; Riggs et al., 1968); the second is the relative weak binding to nonoperator double-stranded DNA (Lin and Riggs, 1972, 1974). This nonoperator DNA binding is extremely important for explaining the fast kinetics of repressor binding to operator and is also important for the in vivo regulation of the *lac* operon (Lin and Riggs, 1974; von Hippel et al., 1974). The genetic analysis of numerous repressor point mutants (Alder et al., 1972; Miller et al., 1975; Müller-Hill, 1975) as well as the biochemical characterization of certain repressor mutants devoid of up to 61 residues at the amino terminus (for a review, see Files et al., 1974) and the properties of the homogeneous tetrameric tryptic core (Platt et al., 1973; Files and Weber, 1976) predict that the amino-terminal end of the repressor molecule is a necessary requirement not only for the specific operator DNA binding but also for the weak nonoperator DNA binding (Lin and Riggs, 1975). Furthermore similar binding mechanisms are used for both operator and nonoperator DNA binding (Lin and Riggs, 1975). We have now shown directly that the headpieces, which span the amino-terminal end of the *lac* repressor, are able to bind nonoperator DNA as judged by the traditionally used nitrocellulose filter assay. However, the headpieces do not have the strong and specific binding to *lac* operator DNA. This latter result again agrees well with previous studies using mixed tetrameric hybrids between normal repressor subunits and subunits deficient in operator binding. Both in vivo (Müller-Hill et al., 1968) and in vitro (Geisler and Weber, 1976) data showed that tetrameric repressors containing one normal subunit and three deficient subunits have lost the strong and specific operator DNA binding. Thus the weak but physiologically important (see above) binding of repressor to nonoperator DNA is retained in the structure of the monomeric headpieces but the strong and specific operator DNA binding, which requires an oligomeric or tetrameric molecule is lost in the monomeric headpiece. Future experiments with mutant headpieces, chemically modified headpieces, and extended physical-chemical characterization of the DNA-headpiece interaction should reveal those structural features of the headpiece involved in nonoperator DNA binding.

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