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Chemical Modification of Arginine Residues in the Lactose Repressor†

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ABSTRACT: The lactose repressor protein was chemically modified with 2,3-butanedione and phenylglyoxal. Arginine reaction was quantitated by either amino acid analysis or incorporation of ^{14}C-labeled phenylglyoxal. Inducer binding activity was unaffected by the modification of arginine residues, while both operator and nonspecific DNA binding activities were diminished, although to differing degrees. The correlation of the decrease in DNA binding activities with the modification of ~1–2 equiv of arginine per monomer suggests increased reactivity of a functionally essential residue(s). For both reagents, operator DNA binding activity was protected by the presence of calf thymus DNA, and the extent of reaction with phenylglyoxal was simultaneously diminished. This protection presumably results from steric restriction of reagent access to an arginine(s) that is (are) essential for DNA binding interactions. These experiments suggest that there is (are) an essential reactive arginine(s) critical for repressor binding to DNA.

Expression of the lactose metabolizing enzymes of *Escherichia coli* is regulated by a repressor protein. This protein binds with high affinity to the operator sequence in the DNA, physically blocking RNA polymerase transcription of these coordinately regulated enzymes (Miller & Reznikoff, 1980). In the presence of inducer molecules, the repressor undergoes a conformational change, which results in diminished affinity for operator DNA. The excess of nonspecific DNA binding sites in the *E. coli* genome can then compete effectively with the operator for repressor binding, and transcription of the mRNA for the *lac* enzymes can be initiated. The affinities of the *lac* repressor for various nonspecific and operator-containing DNAs have been measured under a variety of conditions (deHaseth et al., 1977; Record et al., 1977; Revzin & von Hippel, 1977; Barkley et al., 1981; Winter & von Hippel, 1981; Winter et al., 1981; Whitson & Matthews, 1986; Whitson et al., 1986).

The inability to produce X-ray-quality crystals of the repressor has led to the use of alternative methods in an attempt to delineate structure–function relationships of this protein. In concert with genetic mapping, chemical modification studies provide information regarding functional roles and local environments of individual amino acid residues (Means & Feeney, 1971; Glazer, 1976; Glazer et al., 1975). The roles of specific amino acid residues in the functioning of the lactose repressor have been examined by genetic methods (Müller-Hill et al., 1977; Pfahl et al., 1974; Coulondre & Miller, 1977; Miller, 1979; Miller et al., 1979) and chemical modification (Fanning, 1975; Hsieh & Matthews, 1981, 1985; O'Gorman & Matthews, 1977; Brown & Matthews, 1979; Alexander et al., 1977; Manly & Matthews, 1979; Whitson et al., 1984). The importance of basic residues in DNA binding activity can be deduced from the requirement for the protein to interact with the polyacidic nucleic acid. Basic groups on the surface of the protein must be available to participate in ion pairs with the phosphate groups of the DNA. Lysines-33, -108, and, to a lesser extent, -37 were found to be involved in DNA binding by chemical modification (Whitson et al., 1984), and genetic evidence indicated lysines critical for DNA binding at positions 2, 33, 84, and 290 (Pfahl et al., 1974; Coulondre & Miller, 1977; Miller, 1979; Miller et al., 1978, 1979; Schmitz et al., 1978; Miller & Schmeissner, 1979). Amino acid replacements resulting from missense mutations have indicated that arginines-22, -51, -168, and -303 influence DNA binding activity of the repressor (Miller & Schmeissner, 1979; Miller, 1979). Chemical modification of arginine residues has been employed to provide further insight into the participation of these residues in repressor function. We have modified the lactose repressor with 2,3-butanedione and phenylglyoxal, which specifically react with the guanidinium functional group of arginines, to correlate effects on binding activities with reaction of these residues.

Materials and Methods

Purification of Repressor. The lactose repressor was purified from *Escherichia coli* CSH 46 grown in a 100-L fermentor. Cells were stored frozen at −20 °C until use. Repressor was isolated by the method of Rosenberg et al. (1977) as modified by O'Gorman et al. (1980). The protein yield was approximately 1–4 mg/g of cells. Repressor concentrations were determined spectrophotometrically with an $A_{280}^{1mg/mL}$ = 0.6 (Huston et al., 1974). The purity of the repressor (>95%) was assessed by sodium dodecyl sulfate gel electrophoresis.

Assay of Repressor. Isopropyl β-D-thiogalactoside (IPTG)[1] binding activity was determined by the nitrocellulose filter and

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[1] Abbreviations: bp, base pair(s); DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; IPTG, isopropyl β-D-thiogalactoside; PGO, phenylglyoxal; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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ammonium sulfate precipitation methods described by Bourgeois (1971). Inducer binding was assayed in 0.01 M Tris-HCl, pH 7.4, 0.01 M $MgCl_2$, 0.2 M KCl, 10^{-3} M EDTA, and 10^{-4} M DTT. Nonspecific DNA binding assays were carried out with 2×10^{-11} M ^{3}H-labeled λ*plac* DNA in the presence of IPTG (10^{-3} M), as described by O'Gorman et al. (1980). Stoichiometric operator DNA binding assays using the nitrocellulose filter method were performed as described previously (Riggs & Bourgeois, 1968; Hsieh & Matthews, 1981) with the modifications described by Whitson and Matthews (1986). ^{3}H-Labeled λ*plac* was isolated as described by Whitson and Matthews (1986) and used as the operator DNA (2×10^{-11} M), and binding activities were determined relative to control protein. Repressor concentrations represent total protein concentrations. The protein exhibited 80–100% DNA binding activity under stoichiometric binding conditions. The buffer for kinetic DNA binding experiments was 0.01 M Tris-HCl, pH 7.4, 10^{-4} M DTT, 10^{-4} M EDTA, 5% dimethyl sulfoxide, and 0.15 M KCl (TFB). Bovine serum albumin (50 μg/mL) was added to this buffer to stabilize diluted protein (TBB). K_d determinations were performed in 0.01 M Tris-HCl, pH 7.4, 0.15 M KCl, 10^{-4} M DTT, 10^{-4} M EDTA, 5% dimethyl sulfoxide, and 50 μg/mL BSA, with ^{32}P end labeled 40-bp operator fragment (Whitson & Matthews, 1986). The concentration of 40-bp fragment was 4.6×10^{-12} M.

Protein Gel Electrophoresis. Native discontinuous polyacrylamide gels were performed by the procedure of Laemmli (1970) with the omission of sodium dodecyl sulfate. A Hoefer SE-500 electrophoresis apparatus was used. Gels were electrophoresed for 8–10 h at 60–150 V. Gels were fixed and stained with 0.1% Coomassie Brilliant Blue R in 10% acetic acid–10% 2-propanol for 15–30 min. Destaining was performed in 10% 2-propanol–10% acetic acid.

Reaction of Repressor with 2,3-Butanedione. 2,3-Butanedione was purchased from Sigma, redistilled (bp 88 °C) weekly, and stored in the dark under nitrogen at 4 °C. Protein was dialyzed into 50 mM borate, pH 8.0, purged periodically with nitrogen. Protein (0.9 mg/mL) was reacted with 2,3-butanedione at the indicated molar ratios over repressor monomer concentration. The reaction mixture was incubated in the dark at room temperature. Unreacted reagent was separated from the protein by elution of the sample from a Sephadex G-25 (150 mesh) column (1.4 × 16 cm) equilibrated in 50 mM borate, pH 8.0. Repressor was also reacted with 2,3-butanedione in the presence of ligands. IPTG (10^{-3} M final concentration) and calf thymus DNA (0.1 mg/mg of repressor) were added to separate repressor samples and incubated 5–10 min before the 2,3-butanedione was added. Samples were eluted from the G-25 (150 mesh) column mentioned previously, and inducer and DNA binding activities were determined relative to unmodified protein treated in similar fashion.

Reaction of Repressor with Phenylglyoxal. Phenylglyoxal solutions were prepared fresh from the solid (Sigma, 95% pure). Protein (1–2 mg/mL) was dialyzed into 0.1 M sodium bicarbonate, pH 8.3, purged periodically with nitrogen. Protein (1.0 mg/mL) was reacted with phenylglyoxal at the indicated molar concentration per mole of monomer of repressor. The reaction mixture was incubated 45 min at room temperature and eluted from a Sephadex G-25 (150 mesh) column (1.4 × 16 cm) equilibrated in 0.1 M sodium bicarbonate, pH 8.3, or dialyzed against the same buffer to separate unreacted reagent from the protein. Modified samples were assayed in parallel with control protein, which was treated identically with the exception of the addition of phenylglyoxal.

Phenylglyoxal (PGO) Equivalents Incorporated into the Repressor. [7-^{14}C]Phenylglyoxal (0.27 mg of 25 mCi/mmol) was purchased from Amersham, resuspended in 2 mL of water, and stored frozen. Reactions were carried out with a mixture of labeled and unlabeled PGO. An aliquot was removed from the reaction mixture and counted to determine the total radiolabeled PGO present. After the 30-min reaction, an equal volume of the reaction mixture was filtered on 0.45-μm nitrocellulose and washed with 2 mL of TBB prior to scintillation counting to determine the percent incorporation. From these data, the number of PGO molecules incorporated per protein monomer was calculated. Alternatively, the sample was passed through a Sephadex G-25 (150 mesh) column or dialyzed extensively to remove the unreacted PGO prior to counting of an aliquot of this solution. No significant differences were observed between these methods. Operator DNA binding activities were measured directly on the ^{14}C-labeled protein; however, the incorporated [^{14}C]phenylglyoxal interfered with the determination of nonspecific DNA and inducer binding activities.

Amino Acid Analysis. Protein samples were hydrolyzed with 6 N HCl in vacuo for 20 h at 110 °C. After lyophilization, samples were analyzed on the 5-cm column of a Beckman 120C amino acid analyzer. Extent of modification was determined by the loss of arginine in modified sample compared to that in the control; at low levels of reaction the error in these determinations is quite large as the values are small differences betwen large numbers.

RESULTS

Modification of Repressor with 2,3-Butanedione. 2,3-Butanedione, an arginine-specific reagent, was used to modify the repressor protein. Amino acid analyses of acid-hydrolyzed, modified protein were performed to determine the extent of modification. Results indicated a loss of arginine, with no apparent modification of lysines. Oligomerization of reagent, which decreased reactivity, was reported by Riordan (1973). A comparison of freshly redistilled and reagent-grade 2,3-butanedione indicated a significantly higher reactivity of the *lac* repressor with the freshly redistilled butanedione. Therefore, butanedione was redistilled weekly as described by Petz et al. (1979) and stored at 4 °C.

The optimal pH and borate concentration for butanedione modification were determined for repressor reaction. The effects of pH on butanedione reaction with repressor were determined by both operator DNA binding assay and amino acid analysis of the reacted protein. Essentially no modification or loss in DNA binding activity was observed at pH 7.5. Arginine modification increased with increasing pH (Figure 1). Although the number of arginines modified varied directly with increasing pH, the operator DNA binding activity retained by the modified protein remained constant above pH 8.0. Since only a single equivalent of arginine was modified at pH 8.0 and no further loss of operator binding activity was observed at higher pH, this pH was selected for the remaining studies. Borate is required to stabilize the reacted product; however, Riordan (1973) reported that high concentrations of borate interfered with reaction. Borate concentrations from 25 to 200 mM had little effect on the extent of modification. A borate concentration of 50 mM was arbitrarily chosen for all subsequent reactions.

Reaction of the *lac* repressor with 2,3-butanedione under these conditions (pH 8.0, 50 mM borate) resulted in a time-dependent loss in operator DNA binding and, to a lesser extent, nonspecific DNA binding activities. Reaction was complete by 1 h at all reagent concentrations examined. Increasing the

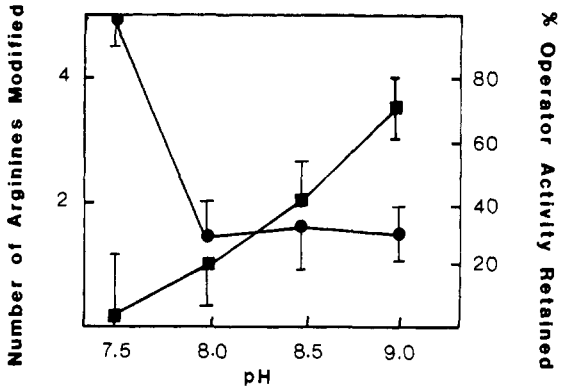

FIGURE 1: Effects of pH on 2,3-butanedione reaction with the repressor. Repressor protein (2.4×10^{-5} M monomer) was reacted with a 300-fold molar excess of butanedione (7.2×10^{-3} M) over monomer for 1 h. Operator binding activity was measured and compared to that of control protein at the same pH. Samples were acid hydrolyzed, and amino acid analysis was utilized to detect the loss of arginine(s). (■) Number of arginines modified per monomer; (●) percent operator activity retained.

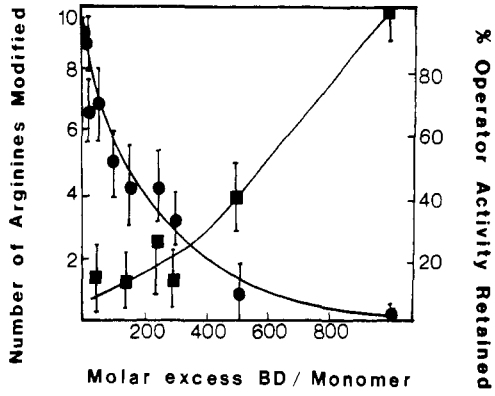

FIGURE 2: Correlation of operator activity loss to arginine modification by 2,3-butanedione. Repressor protein (2.4×10^{-5} M monomer) was modified at the indicated molar excess of butanedione/repressor monomer [(0–2.4) $\times 10^{-2}$ M] for 1 h at room temperature. Operator binding activity was measured by the filter-binding technique, and amino acid analysis was utilized to quantitate the modification of arginine(s). (■) Number of arginines modified per monomer; (●) percent operator activity retained.

molar excess of butanedione per repressor monomer resulted in a decrease in operator DNA binding activity retained (Figure 2). Up to ~2 equiv of arginine was modified at molar excesses up to 300-fold over repressor monomer; however, at low levels of modification the error in these determinations was large because the method involves measuring loss of arginine rather than incorporation of butanedione. A 70% loss of operator DNA binding activity was observed over this same range of reagent concentration. No loss in IPTG binding was observed at molar excesses less than 1000-fold over monomer. The reaction of 2,3-butanedione is reversible; thus, the addition of an excess of arginine to the reaction mixture results in a new equilibrium in which the reagent is displaced from the protein. Operator and nonspecific DNA binding activities were completely recovered within 60 min at the addition of the excess arginine (Figure 3).

Effects of Ligands on 2,3-Butanedione Modification of the Repressor. Modification of the repressor was carried out in the presence of inducer or DNA to determine whether the presence of these ligands affected the loss of activity. IPTG binding activity was unaffected by modification with butanedione in the presence or absence of IPTG (10^{-3} M) or DNA. Operator DNA binding activity, however, was protected approximately 1.5-fold (increase from 48 ± 10% to 73 ± 9% in

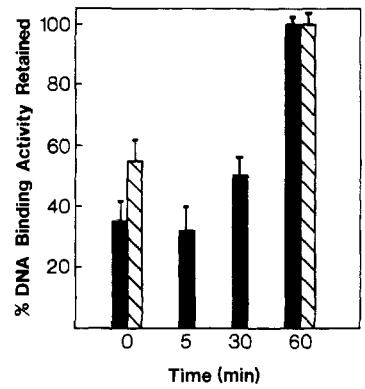

FIGURE 3: Reversibility of 2,3-butanedione modification by the addition of excess arginine. Repressor protein (2.4×10^{-5} M monomer) was modified with a 300-fold molar excess of butanedione (7.2×10^{-3} M) for 1 h before the addition of 1 M arginine (0.1 M final concentration). The time between addition of excess arginine and assay of operator DNA binding activity is indicated. Nonspecific DNA binding activity was also completely recovered with the addition of excess arginine. (Solid bars) Operator DNA binding; (hatched bars) nonspecific DNA binding.

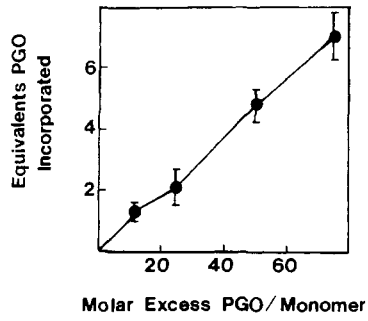

FIGURE 4: Phenylglyoxal modification of the lactose repressor. Repressor (2.7×10^{-5} M monomer) was modified with the indicated excess of phenylglyoxal [(0–2) $\times 10^{-3}$ M] for 30 min. Equivalents of [^{14}C]PGO incorporated per repressor monomer were determined by scintillation counting as described under Materials and Methods.

activity retained) when modification with a 100-fold molar excess was performed in the presence of calf thymus DNA. Protection of arginines could not be confirmed by amino acid analysis due to the large errors in the determination at low levels of modification. Effects of nonspecific DNA binding activity also could not be determined since the calf thymus DNA could not be entirely removed, and its presence interfered with the assay. Inducer presence during reaction did not affect DNA binding loss.

Modification of Repressor with Phenylglyoxal. Phenylglyoxal is also an arginine-specific reagent, but it generates an irreversible product. The number of PGO molecules incorporated was determined with ^{14}C-labeled PGO (Figure 4). No significant changes in inducer binding of the modified protein were observed by either the ammonium sulfate precipitation or the filter-binding methods. Modification of the repressor with phenylglyoxal did, however, decrease operator and nonspecific DNA binding activity (Figure 5). The loss of nonspecific DNA binding activity is less severe than that for operator DNA binding activity at the molar ratios examined. The apparent equilibrium and kinetic constants for binding to ^{32}P-labeled 40-bp operator fragment were determined in 0.15 M KCl TBB at various excesses of phenylglyoxal (Table I). Equilibrium constants determined for each condition were compared to control protein, which had been treated identically. The association rate constant decreased with increasing excesses of phenylglyoxal. The dissociation rate constant, however, was essentially equivalent up to a

Table I: Equilibrium and Kinetic Determinations for Binding Operator and Nonspecific DNA by Phenylglyoxal-Modified Repressor

PGO/repressor monomer	K_{RO} (M)[a]	K_{RO^*} (M)[b]	K_{RD} (M)[c]	k_a (M^{-1} s^{-1})[d]	k_d (s^{-1})[e]
0	1.5×10^{-11}	1.5×10^{-11}	3.5×10^{-8}	8.3×10^{8}	0.015
12.5	3.3×10^{-11}	1.7×10^{-11}	4.7×10^{-8}		
25	5.6×10^{-11}	1.5×10^{-11}	5.8×10^{-8}		
50	9.9×10^{-11}	2.5×10^{-11}	9.3×10^{-8}	3.9×10^{8}	0.015
75	11.4×10^{-11}	4.0×10^{-11}	14.0×10^{-8}	1.5×10^{8}	0.009

[a] K_d of repressor binding to a 40-bp operator fragment in 0.15 M KCl–containing TBB. [b] K_d of repressor binding to a 40-bp operator fragment following phenylglyoxal modification in the presence of calf thymus DNA. [c] K_d for repressor binding to λp*lac* DNA in the presence of 1 mM IPTG. [d] Bimolecular association rate constant for repressor binding to 40-bp operator fragment. [e] Dissociation rate constant for the repressor–40-bp operator complex.

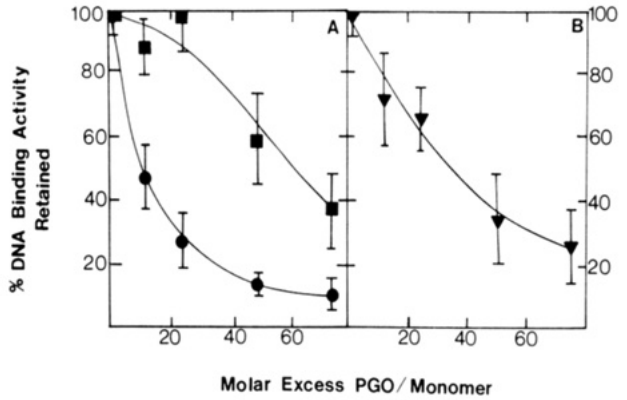

FIGURE 5: Effects of phenylglyoxal modification on DNA binding activity. Repressor (2.7×10^{-5} M monomer) was modified with the indicated excess of PGO [(0–2) $\times 10^{-3}$ M] for 30 min. Activity assays were executed as described under Materials and Methods. (A) Operator DNA binding: (●) operator DNA binding of modified protein; (■) operator DNA binding activity of protein modified in the presence of calf thymus DNA (0.1 mg/mg of protein). (B) Nonspecific DNA binding activity.

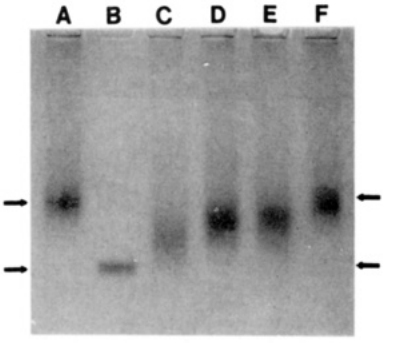

FIGURE 6: Native polyacrylamide gel of phenylglyoxal-modified repressor: (A) repressor; (B) trypsin-resistant core protein of *lac* repressor; (C) repressor modified with a 75-fold molar excess; (D) repressor modified with a 50-fold molar excess; (E) repressor modified with a 25-fold molar excess; (F) repressor modified with a 12.5-fold molar excess. Upper arrows indicate repressor mobility, and lower arrows indicate core protein mobility.

75-fold excess of phenylglyoxal per repressor monomer. Calculated and observed K_d values were in reasonable agreement. A 70% loss of operator DNA activity corresponded to the incorporation of approximately 2 equiv of PGO/repressor monomer. Two phenylglyoxal moieties can react per guanidino group (Takahashi, 1968; Riordan, 1979); thus, a single equivalent of modified arginine may be responsible for this significant loss in operator binding activity. Peptide mapping, utilizing HPLC and radiolabeled PGO, was attempted; however, no ^{14}C-labeled peptides could be detected. The instability of ^{14}C-labeled protein has been observed previously (Jörnvall et al., 1977).

Native Polyacrylamide Gels of PGO-Modified Repressor. The product of PGO modification of arginines is uncharged (Riordan, 1979). Thus, modified protein would be expected to display an altered mobility on a native polyacrylamide gel. PGO-modified protein applied to a 7.5% native polyacrylamide gel migrated slightly faster than repressor but slower than the tryptic core of the lactose repressor, consistent with the modified protein being more acidic than unmodified protein. The broader bands observed for the modified protein suggest that the reaction mixture is not entirely homogeneous, although no unreacted repressor was apparent on the gels (Figure 6).

Salt Dependence of Phenylglyoxal-Modified Protein. The slope of a plot of log K_a vs log [KCl] has been shown theoretically to be related to the number of counterions released upon binding (Record et al., 1976, 1978; deHaseth et al., 1977). Since the modified guanidinium group(s) would no longer be charged and may be involved directly in an ionic interaction, we determined the number of ionic interactions for the DNA complex with protein modified by a 50-fold molar excess (~4.5 Arg modified); the KCl concentration was varied from 0.125 to 0.20 M. The number of ionic interactions for the repressor–40-bp operator fragment was found to be 6.4 ± 0.15, while a value of 5.9 ± 0.30 was determined for the modified protein. These values are not sufficiently different to indicate a significant change in the number of ionic interactions of the modified protein; in addition, residues involved in the interaction with operator may not necessarily be identical for the two species. This protein adaptability was originally demonstrated by Mossing and Record (1985) using various *lac* O^c mutations.

Effects of Ligands on Reaction of Phenylglyoxal with Repressor. Phenylglyoxal reactions with repressor were carried out in the presence of either IPTG or calf thymus DNA. IPTG appeared to have no effect on the activity loss caused by the reagent; in contrast, the presence of calf thymus DNA caused significant alterations in operator DNA binding activity (Figure 5A, Table I). The presence of calf thymus DNA during the reaction significantly protected the operator DNA binding activity up to a 50-fold excess of PGO per repressor monomer. At low molar ratios of reagent, the presence of nonspecific DNA decreased incorporation of radiolabeled PGO; for individual experiments, at 25-fold PGO, incorporation was decreased from 2.3 PGO/monomer to 1.4 by the presence of calf thymus DNA. A similar decrease of ~1 PGO/monomer was observed at 50-fold (3.5 vs 2.1 PGO incorporated). At a 75-fold excess of phenylglyoxal per repressor monomer, the degree of activity and modification protection was decreased. The nonspecific DNA appears to protect arginine residues that are involved in the binding process from modification with PGO at lower reagent excesses.

Discussion

Reaction of the *lac* repressor with 2,3-butanedione or phenylglyoxal resulted in the modification of only arginine residues, as determined by amino acid analysis of the modified protein. Redistillation of butanedione on a weekly basis was

required for reproducible modification of the repressor, as observed previously by Riordan (1973) and Petz et al. (1979). The optimal pH for reaction with the repressor protein was 8.0; reaction at this pH yielded the greatest loss of operator DNA binding activity with the fewest equivalents of arginines modified. Both DNA binding activities of the butanedione- or phenylglyoxal-modified repressors were affected. With the incorporation of 1–2 equiv of butanedione, ~50% of the nonspecific DNA binding activity was retained, while <20% of the operator DNA binding activity remained. Modification of the repressor with phenylglyoxal also resulted in a greater loss in operator DNA binding activity relative to nonspecific DNA binding activity. Previous chemical evidence for overlapping, but independent, nonspecific and operator DNA binding sites has been observed (Hsieh & Matthews, 1981; Alexander et al., 1977; O'Gorman & Matthews, 1977; Manly & Matthews, 1979; Burgum & Matthews, 1978; Hsieh & Matthews, 1985).

Published results have indicated that reaction at high pH followed by assay at pH 7.4 does not affect repressor protein activity (Yang et al., 1977). Although reaction occurs with the conformation of the protein at elevated pH, the assays assess the effect of modified arginines on the protein conformation and interactions at neutral pH. Structural changes consequent to pH increase may be fixed by reaction of arginines in the protein and therefore do not reverse upon lowering of the pH. Although significant effects of pH on inducer binding have been reported (Friedman et al., 1977; Ohshima et al., 1974; Butler et al., 1977; Daly & Matthews, 1986), operator DNA binding activity does not vary significantly with pH from 7.5 to 9.2 at the salt concentrations utilized for assay in this study (Barkley et al., 1981; Daly & Matthews, 1986; P. A. Whitson, unpublished results). If arginine reaction were to trap the protein in its high-pH form, the inducer binding activity would be diminished in concert with effects on ligand binding due to modification of arginines essential to inducer or DNA interaction. The high-pH form of the protein appears to be the preferred form for operator binding (Daly & Matthews, 1986), and trapping the protein in this state would favor DNA binding rather than decreasing operator affinity as observed. In addition to confirming that the reacted protein is not fixed in a high-pH conformation, the absence of effect of modification on inducer activity suggests that any arginine residues in this site are unavailable for reaction. The reversal of butanedione effects by excess free arginine also confirms that no deleterious effects on the protein structure (e.g., denaturation and significant conformational alteration) have occurred during modification and assay. This technique, while limited, provides an alternative approach to genetic methods for detecting participation of specific residues in binding activities of this regulatory protein.

Homology of the *lac* repressor to other DNA binding proteins suggests a helix–turn–helix DNA binding motif within the NH_2-terminal domain (Matthews et al., 1982; Sauer et al., 1982; Weber et al., 1982). NMR studies of the NH_2 terminus alone and complexed with operator fragments confirm such a helical arrangement and suggest Arg-22 may contact the GC-5 base pair in the operator sequence (Zuiderweg et al., 1985; Boelens et al., 1987). Miller (1984) has shown that replacement of Arg-22 by Pro, Cys, Phe, or Leu results in a repressor with an I^{-d} phenotype. Furthermore, Ebright (1986) has suggested contact between the side chain of Arg-22 and the AT-8 base pair. Although the site(s) of arginine modification is (are) indeterminate in our study, the differential effects on specific and nonspecific binding are consistent with Arg-22 involvement.

The reaction of repressor with butanedione or phenylglyoxal in the presence of calf thymus DNA protected the operator DNA binding activity and for phenylglyoxal simultaneously decreased the equivalents of arginine modified. These data indicate that the modified arginines are either directly involved with or near the DNA binding region of the protein, although a structural change upon binding the DNA which makes arginines less available for modification cannot be disqualified as an explanation for these results. The basic nature of the arginine side chain is consistent with the interpretation that the residue(s) protected from modification in the presence of DNA participate(s) directly in protein–DNA interactions by ion pair formation; however, the difference in number of ion pairs for PGO-modified compared to unmodified repressor is not significant. Whether (1) an ionic contact lost is replaced by another in an adaptive response of the protein similar to the model proposed by Mossing and Record (1985) for O^c mutants, (2) the arginine(s) modified play(s) a structural role in maintaining active conformation, or (3) the residue(s) make(s) nonionic contacts with bases in the operator sequence is not apparent from the data. The latter interpretation is consistent with modification of Arg-22 preventing interaction with specific base pairs in the operator. The ability to completely reverse the butanedione modification with concomitant recovery of DNA binding activity indicates that the argine(s) modified is (are) in some fashion crucial for DNA recognition.

Arginines within an active site frequently possess features distinguishing them chemically from other arginines in the protein structure (Borders & Riordan, 1975; Bleile et al., 1975; Borders & Wilson, 1976; Lange et al., 1974; Riordan, 1973). The consequent selectivity in the reaction with modifying agents may be the result of increased reactivity of functionally essential arginine and/or decreased reactivities of other arginines due to solvent–arginine interactions. Modification of the repressor protein with butanedione at low molar ratios (<300-fold) results in a significant decrease in DNA binding with modification of <2 equiv of arginine; above a 300-fold molar excess per monomer, the number of arginines modified increases significantly. Phenylglyoxal modification also affects one to two arginine residues with a corresponding loss of DNA binding capacity. If the equivalents of arginine modified represent the reaction of individual residues on the protein, these data would indicate that while a single arginine may be critical for specific DNA recognition, nonspecific DNA recognition is a more flexible process. In analogy to the thermodynamic evidence for the adaptability of repressor recognition of single base substituted operator fragments (Mossing & Record, 1985), the modified protein may utilize different contacts in order to bind nonspecifically to the DNA. These data indicate that the 1–2 equiv of arginine modified initially possesses unique reactivity distinguishing them from the remaining 17–18 arginine residues and suggest critical involvement of the modified arginines in the process of DNA recognition.

Registry No. Arg, 74-79-3; lactose, 63-42-3.

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