In order to elucidate the role of the aromatic ring in recognition of the sugar ring, Trp-62 of hen egg white lysozyme, which is proposed on the basis of x-ray crystallography data to make contact with a sugar ring through van der Waals interactions, was replaced with aliphatic amino acids (Leu, Ile, Val, and Ala) and Gly by site-directed mutagenesis. In spite of the loss of the aromatic effect, these mutant lysozymes, except for the Trp-62 → Gly mutant, showed higher bacteriolytic activity than the wild-type lysozyme. Furthermore, the Trp-62 → Gly mutant still retained appreciable bacteriolytic activity.

On the other hand, by these replacements, the enzymatic activities toward non-charged substrates were markedly reduced. Additionally, the side-chain structure of position 62 was found to be largely responsible for recognition of a saccharide ring in its active site cleft. NMR analysis of the Trp-62 → Leu and Trp-62 → Gly mutants indicated that the structural effects of Trp-62 replacements were localized in the loop region around position 62 and the part of the β-sheet containing the hydrogen bonding network important for enzymatic activity.

Thus, we conclude that Trp-62 not only interacts with oligosaccharide through van der Waals contact, but also maintains the local structural conformation to produce the lysozyme-oligosaccharide interaction.

In carbohydrate-protein interaction, it has been pointed out (Quiocco, 1986, 1988; Vyas, 1991) that a network of hydrogen bonds containing water molecules is very important for carbohydrate specificity; in addition, van der Waals contact of aromatic side chains on the saccharide ring is generally observed (Trp-17 of arabinose-binding protein, Tyr-404 of glycogen phosphorylase (Johnson et al., 1988) and Trp-340 of maltodextrin-binding protein (Spurlino et al., 1991), and so forth).

Hen egg white lysozyme (EC 3.2.1.17) catalyzes the hydrolysis of β-1,4 glycosidic bonds of polysaccharides that are alternating polymers of GlcNac and MurNAc2 or homopolymers of GlcNac (chitin) (Jolles and Jolles, 1984). The three-dimensional structure of hen egg white lysozyme has been determined by x-ray crystallography (Blake et al., 1967) and recently by two-dimensional 1H nuclear magnetic resonance methods (Smith et al., 1993). In the active site cleft of this enzyme, three tryptophans, Trp-62, -63, and -108, are proposed to be involved in substrate binding on the basis of x-ray crystallographic data (Fig. 5) (Imoto et al., 1993). In the binding of the substrate analogue to the active site of the lysozyme, the indole ring of Trp-62 is observed to show considerable induced-fitting and to make extensive contact with the non-polar surface of the sugar residue in substrate B (Cheetham et al., 1992). Therefore this aromatic ring-sugar interaction is one of the general features of carbohydrate-protein interaction described above. The tryptophan at this position is replaced with tyrosine in homologous lysozymes from human, rat and bovine. Replacements of this residue with other aromatic amino acid residues by site-directed mutagenesis on the basis of molecular evolution (Kumagai and Miura, 1989; Kumagai et al., 1992, 1993) have been observed to produce marked changes of the activity of the enzyme. Selective chemical modifications disrupting the indole ring of Trp-62 have also led to drastic reduction of bacteriolytic activity of hen lysozyme (Hayashi et al., 1965; Yamazaki et al., 1976).

In the present paper, to elucidate the effect of aromatic residues at position 62 on enzymatic activity, Trp-62 was replaced by site-directed mutagenesis with non-aromatic amino acids (Leu, Ile, Val, Ala, and Gly) containing no reactive group on their side chains. We found that the side chain at position 62 is not critical for the bacteriolytic activity of hen egg white lysozyme, although these replacements reduced the enzymatic interaction with non-charged substrates. Furthermore, two-dimensional 1H NMR analysis of two mutant lysozymes was performed in order to reveal the structural effects of these mutations.

**EXPERIMENTAL PROCEDURES**

Rents—Restriction endonucleases, DNA-modifying enzymes, and DNA-sequencing kits were purchased from Takara Shuzo (Kyoto, Japan) and Toyobo (Osaka, Japan). Microcococcus lysodeikticus cells were from Sigma. Hen egg white lysozyme, glycyl chitin, tri-N-acetyl-chitosan, (GlcNAc)3, and p-nitrophenyl pentamers-N-acetyl-β-p-chitopentaoside (PNP-GlcNAc)6 were purchased from Seikagaku Kogyo (Tokyo, Japan). The high performance liquid chromatography column (YMC-Pac A-014, 6 × 300 mm) was from Nihon Kogyo (Tokyo, Japan). S-Sepharose (fast flow) and Mono S H5/5 were obtained from Pharmacia. All other chemicals used were of reagent grade appropriate for biochemical use.

Production and Purification of Mutant Hen Lysozymes—Mutant hen lysozyme cDNAs used in this study were prepared by site-directed mutagenesis as described previously (Kumagai and Miura, 1989; Kumagai et al., 1992). The mutations were confirmed by DNA sequencing analysis. According to the procedures of Kumagai and Miura (1989), expression and purification of mutant hen lysozymes were carried out using the expression system in Saccharomyces cerevisiae AR22. Mutant
lysozymes secreted into yeast growth medium were purified by cation-exchange chromatography on S-Sepharose (fast flow) followed by a Mono S HR 5/5 column (Archer et al., 1990) or an affinity chromatography using chitin-celite column (Yamada et al., 1985). The lysozyme preparation was loaded onto the chitin-celite column (12 x 875 mm) equilibrated with 0.1 M sodium acetate buffer containing 0.5 M NaCl (pH 5.5). The lysozyme was eluted by applying a pH gradient from the starting buffer to 1 M CH₃COOH. The size and homogeneity of the purified mutant enzymes were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970). The enzyme concentration was determined spectrophotometrically (E280 nm = 26.4 for the wild-type hen lysozyme), and the molar absorption coefficients of position 62 mutant lysozyme were adjusted by a factor of 0.85 (Kumagai and Miura, 1989).

**Enzymatic Activities of the Mutant Hen Lysosomes**—Bacteriolytic activity of lysozyme was assayed by the method of Parry et al. (1965) with slight modification (Kumagai et al., 1992). Lysozyme was added to 1 ml of a suspension of *M. lysodeikticus* cells in 50 μM sodium phosphate buffer (pH 6.2) at 25°C. The decrease in absorbance at 540 nm after 1 min was measured and compared with the wild-type and mutant lysosomes. The pH dependence of bacteriolytic activity of the wild-type and Trp-62 mutant lysosomes were determined at 25°C and ionic strength 0.1 M in the same manner. According to the method of Miller and Golder (1960), buffers which have constant ionic strength (I = 1) were prepared (acetic acid buffer (pH 4.2–5.7), phosphate buffer (pH 6.0–7.6), and Veronal buffer (pH 8.0–9.0)) were used. Hydrolytic activity toward glycol chitin was measured following the reducing group procedure (Imoto and Yagishita, 1971). Glycol chitin (0.05% w/v) was incubated with or without lysozyme at 25°C for 24 h in 20 mM sodium acetate buffer (pH 5.0). The ionic strength was adjusted to 0.1 M NaCl.

Constants of binding to tri-N-acetylchitotriose were determined by the fluorescence method (Kumagai et al., 1992). The fluorescence spectra of enzyme-inhibitor complex were measured in 50 mM Tris-HCl (pH 7.5) and 0.1 M NaCl at 25°C, using a Hitachi 8500 fluorescence spectrophotometer.

Hydrolytic activity toward PNP-(GlcNAc)ₙ was measured by the method of Nanjo et al. (1988) as refined by Kumagai et al. (1993). PNP-(GlcNAc)ₙ (0.26 mM) was incubated with 1 μg of each lysozyme at 37°C in 50 μl of 50 mM sodium acetate buffer, pH 5.0. The reaction products, PNP-(GlcNAc)ₙ (n = 1–5), were analyzed by high performance liquid chromatography, using a YMC-Pac A-014 (4 x 300 mm) column, and detected at 230 nm. The concentration of each GlcNAc derivative was determined by calculating the area of each eluted peak. Under these conditions, linearity of the reaction time course was observed with a decrease of PNP-(GlcNAc)ₙ and an increase of PNP-GlcNAc and PNP-(GlcNAc)ₙ up to 45 min.

**Structural Analysis of Hen Lysosomes**—by NMR—H NMR experiments were performed at 500 MHz on a Bruker AMX 500 spectrometer at 37°C. All samples were studied in H₂O containing 10% D₂O to provide the field/frequency lock signal. The wild-type lysosome sample contained 4 μM in a volume of 400 μl at pH 4. The Trp-62 → Leu and Trp-62 → Gly samples contained about 0.5 μM in a volume of 200 μl at pH 4. Proton chemical shifts were referenced with respect to the water signal, which is 4.64 ppm downfield from the proton resonance of 4,4-dimethyl-4-silapentane-1-sulfonate at 37°C.

The two-dimensional NMR experiments, 512 free induction decays of 2000 data points were collected by the time proportional phase incrementation method (Ernst et al., 1987), and spectra of 1000 x 2000 real data points were obtained with zero-filling, after which a two-dimensional Fourier transformation with 1/4 and 1/2 shifted sine-bell window functions for t₁ and t₂ dimensions, respectively, was performed. Double quantum-filtered two-dimensional correlation spectroscopy (DQF-COSY) was performed according to Rance et al. (1983). The assignments of the two-dimensional homonuclear Hartmann-Hahn spectroscopy (HOHAHA) measurement (Davis and Bax, 1985; Bax and Davis, 1985; Griesinger et al., 1988) was about 70 ms, and that for two-dimensional NOE spectroscopy (NOESY) measurement (Ernst et al., 1987) was 150 ms.

**Assignment of 1H NMR Signals**—The 1H NMR signals of the wild-type lysozyme have been assigned (Rodfield and Dobson, 1988). Assignments for position 62 mutant lysozymes were obtained by comparing the C'H-NH fingerprint region of the DQF-COSY spectra of these proteins with that of the wild-type lysozyme at the same temperature. Peaks at the same positions were initially assigned to originate from corresponding protons in the two proteins. These assignments were confirmed and extended to residues in which protons had different chemical shifts from the wild-type lysozyme using standard sequential assignment methods (Wuthrich, 1986) and comparison of HOHAHA and NOESY spectra of the wild-type and position 62 mutant lysozymes.

**RESULTS**

**Preparation of Mutant Hen Lysosomes**

All position 62 mutants were obtained and purified, as confirmed by analyzing the SDS-polycrylamide gel electrophoresis data. Fig. 1A shows an elution pattern of the Trp-62 → Leu mutant lysozyme obtained by chitin-celite column chromatography. Whereas the wild-type lysozyme was eluted from the chitin-celite column almost at 1 M CH₃COOH (data not shown), consistent with the previous result reported by Yamada et al. (1985), the Trp-62 → Leu mutant enzyme was found to elute at a much lower CH₃COOH concentration. By applying this affinity chromatography, the contaminating materials of low molecular weight were removed, and the mutant lysozyme was purified to homogeneity, judging from the data of SDS-polycrylamide gel electrophoresis (Fig. 1B).

The mutations were confirmed by DNA sequencing. The Trp-62 → Leu and Trp-62 → Gly mutants were subjected to 500-MHz 1H NMR spectroscopic analysis, and the replacement of Trp-62 was again confirmed by observing the disappearance of the N1H proton of Trp-62.

**Enzymatic Characterization of Mutant Hen Lysosomes**

The enzymatic activities of mutant hen lysozymes were assayed using glycol chitin, PNP-(GlcNAc)₉, and *M. lysodeikticus*...
Role of a Tryptophan in Active Site of Lysozyme

TABLE I
Bacteriolytic activities and hydrolytic activities toward glycol chitin of wild-type and position 62 mutant lysozymes

<table>
<thead>
<tr>
<th></th>
<th>Bacteriolytic activities</th>
<th>Hydrolytic activities</th>
<th>Binding constant to (GlcNAc)3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trp-62 (wild-type)</td>
<td>100</td>
<td>100</td>
<td>7.5 \times 10^4</td>
</tr>
<tr>
<td>Trp-62 → Leu</td>
<td>250</td>
<td>6</td>
<td>1.8 \times 10^7</td>
</tr>
<tr>
<td>Trp-62 → Ile</td>
<td>200</td>
<td>8</td>
<td>1.8 \times 10^7</td>
</tr>
<tr>
<td>Trp-62 → Val</td>
<td>220</td>
<td>5</td>
<td>1.7 \times 10^7</td>
</tr>
<tr>
<td>Trp-62 → Ala</td>
<td>170</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Trp-62 → Gly</td>
<td>50</td>
<td>&lt;1</td>
<td>-</td>
</tr>
</tbody>
</table>

* N.C. measured.

In the presence of Trp-62 from the decrease of PNP-(GlcNAc)3. The ratios of substrate-BNP-GlcNAc and PNP-(GlcNAc)2. Thus we assumed that PNP-(GlcNAd5 binds to lysozyme in two ways (binding modes 1 and 2, Fig. 3), through which PNP-GlcNAc and PNP-(GlcNAc)2 are generated, respectively (Kumagai et al., 1993). Table II summarizes the hydrolytic activities toward PNP-(GlcNAc)3 of position 62 mutant lysozymes. Total activities were determined from the decrease of PNP-(GlcNAc)3. The ratios of substrate-binding modes 1/2 were found to differ among the mutant enzymes in which Trp-62 was replaced. For example, the ratio of PNP-GlcNAc and PNP-(GlcNAc)2 formation by the Trp-62 → Leu mutant enzyme was almost the same (4.0) as that of the wild-type enzyme. On the other hand, that of the Trp-62 → Ala mutant enzyme was lowered (0.7). Furthermore, it was noteworthy that Trp-62 → Leu and Trp-62 → Val, which differ only in the presence or absence of a β-branched side chain, showed different substrate-binding modes, whereas Trp-62 → Ile and Trp-62 → Val, which are β-branched, exhibited similar ones. These results suggest that a slight difference in the structural features of amino acids at position 62 produces a marked change in binding mode.

In the pH profile of bacteriolytic activity (Fig. 2), the wild-type, Trp-62 → Tyr, and Trp-62 → Phe lysozymes were found to maintain high bacteriolytic activity over a wide pH range (pH 5.2–7.6). However, the bacteriolytic activity of the Trp-62 mutant lysozymes, which did not have an aromatic ring at position 62, showed an optimum pH around 5 and reduced activity at neutral and alkaline pH. On the other hand, Table I shows that the bacteriolytic activity of the mutant hen lysozymes in which Trp-62 was converted to alkylphatic amino acids was higher than that of the wild-type lysozyme at pH 5.2; the Trp-62 → Leu mutant lysozyme in particular exhibited 2.5-fold higher lytic activity. Furthermore, the Trp-62 → Gly mutant protein, which has no side chain of position 62, had appreciable lytic activity (50% of that of the wild-type lysozyme). Thus, in contrast with the enzymatic activities toward non-charged substrates, Trp-62 of hen lysozyme was not essential for bacteriolytic activity.

Structural Analysis of Position 62 Mutant Hen Lysozymes by NMR Measurement

In order to obtain information about the structural effects of the replacement of Trp-62, which was found to affect both substrate specificity and catalytic activity, we measured the 500-MHz proton NMR spectra of the wild-type and two position 62 mutant lysozymes at 37 °C and pH 4. The NMR spectrum of the wild-type lysozyme was identical to that reported by Redfield and Dobson (1988). From analysis of the DQF-COSY and HO-HAHA spectra of the Trp-62 → Gly and Trp-62 → Leu mutant lysozymes (Fig. 4), the majority of proton resonances in these two mutant lysozymes were similar to those in the wild-type lysozyme, and so the main-chain proton resonances of more than 100 amino acid residues were assigned according to Redfield and Dobson (1988). Also, the sequential and secondary structural NOEs in the Trp-62 → Gly and Trp-62 → Leu mutants were essentially identical to those in the wild-type lysozyme (data not shown). In the high field region (~2.5 ppm ~ 1 ppm) the proton resonances of amino acid residues composing the hydrophobic box were hardly shifted in these mutant enzymes. These data indicated that the structures of position 62 mutant lysozymes were similar to that of the wild-type lysozyme.

Hem egg white lysozyme has 6 tryptophan residues (Fig. 5). Table III summarizes the changes in the chemical shift of the ring proton resonances of 5 tryptophans in the Trp-62 → Leu and Trp-62 → Gly mutant lysozymes. Of these tryptophans, 2 residues (111 and 123), which were distant from the active site and components of the hydrophobic core, showed no change in chemical shift upon replacement at position 62. In the case of Trp-108, the N1H and C2H resonances were shifted although the other ring proton resonances were not. On the other hand, almost all ring proton resonances of Trp-63, which is located next to position 62, showed changes in chemical shift. These results indicate that the structural differences among the wild-
Fig. 3. Two productive binding modes of an oligosaccharide substrate in the active site cleft of hen egg white lysozyme. A, B, C, D, E, and F show the subsites in the active site of the lysozyme. The arrow indicates the cleavage site of the substrate. Trp-62, which was replaced by other amino acids in this study, is also represented.

Fig. 4. Comparison of the chemical shifts of the main-chain protons between the Trp-62 → Gly or Trp-62 → Leu mutant lysozyme and wild-type lysozyme. The open boxes indicate the degrees of chemical shift differences of NH and CaH. Asterisks indicate chemical shift changes larger than 0.3 ppm. The solid lines around 0 ppm show no chemical shift difference. No indication means unassigned protons in the present study.

Type and position 62 mutant lysozymes are localized around the active site cleft.

The chemical shift changes in Trp-63, Trp-108, and Trp-28 differed between the Trp-62 → Leu and Trp-62 → Gly mutants, indicating that the changes in chemical shift were not due simply to the loss of the ring current effect of Trp-62 (Table III). In particular, the signal of Trp-28 C5H, which is located in a hydrophobic box adjacent to the saccharide-binding site, was changed by introduction of Gly, but not Leu, at position 62. Thus the replacement of Trp-62 with either Leu or Gly had different influences on the active site conformation of the neighboring Trp-63, Trp-108, and Trp-28, and this would lead to the difference in enzymatic characteristics between the mutants.

Trp-62 → Gly Mutant Lysozyme—We found that some of major chemical shift changes (>0.05 ppm) were observed at the main chain NH of Tyr-53 and Ile-58, the main chain C-H of Ser-50, Asp-52, Ile-58, Asn-59, and Ser-60, and the side chain protons of Ile-58 and Asn-59, which were located in part of the three-stranded anti-parallel β sheet (50-60) containing a network of hydrogen bonds (Asp-52, Asn-59, Ser-50, Asn-46, and Asp-48) important for enzymatic activity (Strynadka and James, 1991). We also observed that other changes at the main chain NH of Gly-62, Trp-63, Asp-66, Gly-71, Asn-74, and Cys-76, the main-chain C=H of Gly-62, Trp-63, Cys-64, Thr-69, and Gly-71, and the side-chain protons of Thr-69, which are located in the loop region (61-78) containing position 62. Some proton resonances disappeared or broadened, and these included Arg-61, Leu-75, and Ile-78, which are located in the loop region (Figs. 4 and 5).

Trp-62 → Leu Mutant Lysozyme—The protons with changes
(>0.05 ppm) in chemical shift lay mainly in residues located in part of the three-stranded anti-parallel β-sheet (50–60) and loop region (61–78), which were almost the same as those observed in the Trp-62 → Gly mutant lysozyme. These were the main chain NH of Ser-50 and Ser-60, the main-chain CH of Ser 50 and the side-chain protons of Asn-59 in the anti-parallel β-sheet, and the main chain NH of Cys-64, Asn-65, Asp-66, and Gly-71, the main-chain CH of Gly-71 and the side-chain protons of Thr-69. The protons which disappeared or broadened included Ile-58, Arg-61, Leu-62, Trp-63, Asn-74, and Leu-75 (Fig. 5). As compared with the Trp-62 → Gly mutant lysozyme, the Trp-62 → Leu mutant had a smaller region, which included the residues whose chemical shifts were changed. For example, the main chain protons of Tyr-53, Cys-76, and Ile-78 showed changes in chemical shift in the Trp-62 → Gly mutant lysozyme, but were hardly shifted in the Trp-62 → Leu mutant lysozyme.

**DISCUSSION**

The enzymatic activities of position 62 mutant hen egg white lysozymes toward non-charged substrates were markedly reduced, and the conversion of Trp-62 to a non-aromatic amino acid also decreased the energy of binding with (GlcNAc)5, which binds the A, B, and C subites in the active site of the wild-type lysozyme, by about 2.2 kcal/mol, but even conversion to other aromatic amino acids, Tyr and Phe, produced an appreciable decrease in the aromatic ring and carbohydrate, but also to proline also decreased the energy of binding with (GlcNAc)5, which hardly shifted in the Trp-62 → Leu activity. Replacement of Trp-62 slightly changed the critical steric configuration around position 62. Detailed analysis of hydrolytic activity toward the synthetic substrate, PNP-(GlcNAc)5, demonstrated that replacement of Trp-62 with non-aromatic amino acids seemed to allow looser modes of substrate binding to the active site of this lysozyme (Table II). The substrate binding mode and active site structure were changed by whether the side chain at position 62 was Cβ-branched (Val and Ile) or not (Leu). Then the slight difference in the structural features of amino acids at position 62 induces the marked changes in the substrate-binding modes.

In spite of the loss of aromatic effects at position 62 in lysozyme, some mutants (Trp-62 → Leu, Trp-62 → Ile, Trp-62 → Val, and Trp-62 → Ala) exhibited higher bacteriolytic activity at pH 6.2 than the wild-type enzyme. The Trp-62 → Gly mutant, which had no side chain, showed appreciable activity (50% of that of wild-type enzyme). Bacteriolyis involves a number of complex factors: 1) the complexity of bacterial cell wall from M. lysodeikticus, in which lysozyme practically hydrolyzes MurNAc-(81–4)-GlcNAc, 2) pH, 3) salt concentration, and so on. However, we suggest that a side chain at position 62 is not essential for bacteriolytic activity, in contrast to the presence of an aliphatic amino acid at position 62, which is favorable for this activity.

Two-dimensional NMR analysis showed that the Trp-62 → Leu and Trp-62 → Gly mutants maintained their native secondary and tertiary structures by confirming the presence of d_{NN} and d_{AN} NOEs and the characteristic peaks of the amino acids composing the hydrophobic core. The regions where the chemical shifts of the proton resonances of position 62 mutants were changed relative to that of the wild-type enzyme were located in the loop region (residues 61–78, containing Trp-62) and in part of the three-stranded β-sheet (residues 50–60, containing a network of hydrogen bonds: Asp-52, Asn-55, and Ser-50). Because the direct ring current effects reach only the areas located in the immediate vicinity, most of the observed changes in chemical shift are thought to be due to local conformation changes. Interestingly these regions in which the structural changes induced by these mutations were observed were coincident with those induced by addition of a substrate analogue, (GlcNAc)_5, as revealed by NMR analysis.² Fukamizo et al. (1992) reported that the signal of Trp-28 CSH, which is not in the active site but in a hydrophobic box adjacent to it, was affected most markedly by adding (GlcNAc)_5 and its analogues, and that the change in this signal was due to loss of a side chain at position 62 (Table III). Thus replacement of Trp-62 slightly affected the active site conformation.

Within the loop region (residues 61–78), a subregion (63–76) has also been found by x-ray analysis to be structurally changed upon binding of a substrate analogue (Strynadka and James, 1991). This region has no direct contact with the carbohydrate substrate and packs against another subregion (59–64) which does make contact with the substrate. Ueda et al. (1990) reported the effect of chemical modification at Trp-62 on protein folding, and Miranker et al. (1991) also speculated from recent NMR analysis that Trp-62, especially through Trp-62/ ² K. Maenaka, K. Kawai, K. Watanabe, F. Sunada, and I. Kumagai, unpublished data.
Trp-63 interaction, may influence local folding. On the other hand, on the basis of x-ray and NMR analysis of oxindolealanine 62 lysozyme, which has lower bacteriolytic activity, Blake et al. (1981) pointed out the importance of Asn-59 for the enzymatic activity. The NH$_2$(6) of Asn-59 is thought to be hydrogen-bonded to the O(6) of the catalytic residue Asp-52 in the hydrogen-bonding network (Strynadka and James, 1991). We observed changes in the chemical shift of both the main-chain and side-chain protons of Asn-59, and by tentative assignment, the changes in chemical shift of NH$_2$ of this residue were found to be distinct among Trp-62 mutants (Trp-62 → Leu, Trp-62 → Gly, Trp-62 → His, and Trp-62 → Phe). Furthermore, other differences in structural changes between Trp-62 → Leu and Trp-62 → Gly (Fig. 4, Table III), which were localized in the active site as described above, were observed. Then our NMR analysis indicated that the introduction of non-aromatic amino acids at position 62 caused subtle perturbation of the local structure packing (residues 61–75), and that the slight conformational difference in the side chain of the catalytic residue Asp-52 was possibly responsible for the effect on the enzymatic activity. Thus, the alterations in the enzymatic properties observed with these mutant lysozymes were determined not only by van der Waals contact between the aromatic ring and the sugar, but also by the precise steric configuration of tryptophan and this surrounding local structural conformation. These results support the idea of Vyas (1991) and Quiocho (1986, 1988) that, in carbohydrate-protein interaction, tryptophan is involved in the binding of substrate carbohydrate through steric hindrance and/or by creation of a suitable nonpolar environment.

REFERENCES


