In this paper, we show that amino acids Glu \textsuperscript{73} and Asp \textsuperscript{75} of staphylococcal nuclease cooperate unequally with Glu \textsuperscript{75} to stabilize its structure located between the C-terminal helix and \( \beta \)-barrel of the protein. Amino acid substitutions E73G and D77G cause losses of the catalytic efficiency of 24 and 16\% and cause thermal stability losses of 22 and 26\%, respectively, in comparison with the wild type (WT) protein. However, these changes do not significantly change global and local secondary structures, based on measurements of fluorescence and CD \( \lambda = 222 \) nm. Furthermore, x-ray diffraction analysis of the E75G protein shows that the overall structure of mutant and WT proteins is similar. However, this mutation does cause a loss of essential hydrogen bonding and charge interactions between Glu\textsuperscript{75} and Lys\textsuperscript{73}, Tyr\textsuperscript{75}, and His\textsuperscript{121}. In experiments using double point mutations, E73G/D77G, E73G/E75G, and E75G/D77G, significant changes are seen in all mutants in comparison with WT protein as measured by fluorescence and CD spectroscopy. The losses of thermal stability are 47, 59, and 58\%, for E73G/D77G, E73G/E75G, and E75G/D77G, respectively. The triple mutant, E73G/E75G/D77G, results in fluorescence intensity and CD \( \lambda = 222 \) nm close to those of the denatured state and in a thermal stability loss of 65\% relative to the WT protein. Based on these results, we propose a model in which significant electrostatic interactions result in the formation of a locally stable structure in staphylococcal nuclease.

The diversity of protein sequences and the limited instrumentation for tracking the process of protein folding from a denatured state have left the problem of protein folding unsolved. The “Levinthal paradox” \( (1) \) is still an unresolved issue for protein researchers. With rigorous investigation, however, both the kinetics and thermodynamics of protein folding may yield answers to this major biological question. From the kinetic point of view, a protein can be refolded in vitro to its active three-dimensional conformation on a millisecond time scale, as we have shown for staphylococcal nuclease (SNase) \( (2-4) \). The sequence of equilibrium reactions among three denatured states and one native state has been established \( (5) \):

\[
D_1 \rightleftharpoons D_2 \rightleftharpoons D_3 \rightleftharpoons N_0,
\]

where \( D_i \) (\( i = 1, 2, 3 \)) denotes the protein in its unfolded state and \( N_0 \) is the protein in its native state. Under this scheme, a least activation path model for protein folding was established and used to help to solve puzzles such as accumulated intermediates, random searches among “microscopic states” \( (6) \), and the cofactor roles of chaperonin proteins such as prolyl isomerase, disulfide isomerase, and molecular chaperones \( (7-9) \). Recently, the chaperonin function of the GroEL-GroES-ATP system \( (10) \) in the forced unfolding of ribulose-1,5-bisphosphate carboxylase-oxygenase was reported \( (11) \). Regardless of whether protein refolding is done with or without the assistance of chaperones, rigorous kinetic analysis is necessary to understand protein folding \( (12, 13) \). The recent development of photolabile linkers has made the study of the early events of protein folding possible, although protein refolding may be completed within a matter of nanoseconds or less \( (14, 15) \).

Protein folding is also governed by thermodynamics, since it has been observed that an unfolded protein can spontaneously refold to its native structure at the global free energy minimum \( (5, 16, 17) \). This observation illustrates the basis of the Levinthal paradox \( (1) \), which notes that the number of possible conformational states of a protein can be enormous and that it can take an infinitely long time to find the energy minimum that represents the native state. Although thermodynamic measurements cannot address the questions of rates and pathways of protein folding, a protein's energy state can be expressed as an aggregate thermal unfolding energy that is locally additive: e.g. \( \Delta H_{\text{unfolding}} = \sum \Delta H_i \) (\( i = 1, 2, 3, \ldots, n \)), where \( \Delta H_i \) is the energy released by folding of a locally stable segment. The locally maintained energies are much smaller than those energies produced by covalent bonding. Therefore, a protein’s local native structures are only marginally stable in solution. Despite this, they remain stable in the face of relatively severe perturbations although only weak forces such as electrostatic interactions, van der Waals forces, hydrogen bonding, and hydrophobic interactions contribute to their local structures. Quantitative determination of the energetics of protein unfolding using point mutations is a promising approach to addressing the problem of protein folding. In this paper, we used wild type SNase and seven mutants, E73G, E75G, D77G, E73G/E75G, E73G/D77G, E75G/D77G, and E73G/E75G/D77G, to test in part the above thermal scheme. A local stable segment surrounded by Glu\textsuperscript{73}, Glu\textsuperscript{75}, and Asp\textsuperscript{75} may exist, and these residues may contribute significantly to SNase stability by maintaining the native conformation of the whole protein. X-ray structural analysis of E75G (Fig. 1) was done, and the results further support the arguments regarding the loss of
into 2 ml of LB medium containing ampicillin, and the culture was
containing WT or mutant SNase expression plasmids were streaked on
medium. These flasks were again incubated in the shaker incubator at

37°C of LB medium. The flask was incubated in the shaker incubator at

then poured into each of five 1-liter flasks containing 400 ml of LB

The overall ribbon diagram is shown. The resolution is 2.2 Å. The side chains of Lys9, Tyr93, Gly75, and His121 are indicated in the figure.

Both Ca2+ and pdTp are labeled and shown using a ball-and-stick model. The diagram reveals the relative position of the residues around position 75 and the active sites.

stability and enzyme activity after the replacements of Glu or Asp by Gly in the local area of SNase between C-terminal α-helix and a β-barrel.

**EXPERIMENTAL PROCEDURES**

**Materials**—The WT staphylococcal nuclease nuc gene was obtained from Prof. Tian Yow Tsong and originally donated by Prof. David Shortle. Luria-Bertiani broth and isopropyl-1-thio-β-D-galactopyranoside were purchased from Difco Laboratories and Sigma, respectively. Salmon testes DNA and some analytical grade chemicals such as EDTA, Tris-HCl, CaCl2, NaCl, and mineral oil were obtained from Sigma. Substrate DNA was used without further purification. Guanidine hydrochloride and dNTPs were purchased from Roche Molecular Biochemicals. Absolute ethanol (>99%) was obtained from Panreac, and urea was a product of Acros. The Stratagene QuickChange™ kit containing Pfu DNA polymerase, × 10 reaction buffer, and DpnI restriction enzyme was purchased from Stratagene. 2-Methyl-2,4-pentanediol was purchased from Merck. Water used for these experiments was deionized and distilled.

**Site-directed Mutagenesis**—Mutants used for this experiment were generated by the PCR site-directed mutagenesis method. A commercial kit (Stratagene QuickChange™) was used for sample preparation. Glu75, Glu77, and Asp77 were replaced by Gly. Plasmid pTrc-99A encoding wild type (WT) SNase was stored at -20 °C before mutagenesis. Two complementary 33-mer primers that included the Gly codon at positions 1–29 were purchased from Dharmacon (Carboxy-Methyl-25). The column was loaded with protein solution and washed with buffer B. The sample was eluted with buffer C (6 ìM urea, 1 ìM EDTA, 1 M NaCl, 50 mM Tris-HCl, pH 9.2) and collected using a fraction collector measuring absorbance at 280 nm. The purified proteins were dialyzed with distilled water and then lyophilized using a vacuum freeze dryer. The yield of protein was calculated. The purity was checked by 15% SDS-PAGE with Coomassie Blue stain. A purity of over 90% was estimated by densitometry (Molecular Dynamics, Inc., Sunnyvale, CA). The protein extinction coefficients were calculated with Gill and von Hippel’s method (18).

**Enzyme Activity Assay**—Enzyme activity was determined by incubating the enzyme with a DNA substrate and measuring the change of absorbance at 260 nm. 50 ìg of salmon sperm DNA were dissolved in 1 ml of buffer (10 mM CaCl2, 25 mM Tris-HCl, pH 8.8). The mixture was boiled for 30 min and then cooled on ice for 15 min. 5 ìl of enzyme (1 mg/ml) were gently mixed with 1 ml of substrate buffer in a spectrophotometer cuvette. The change in absorbance at 260 nm was recorded as a function of time. Nonlinear regression analysis was used to calculate the Michaelis constant and the initial velocity. The data were evaluated using Enzfitter software (Biocraft, Toyama, Japan). The turnover rate of the enzyme, kcat, was calculated as Vmax/[Eo], where [Eo] is the initial concentration of the enzyme. The ratio kcat/Km was used to determine catalytic efficiency.

**CD Spectroscopy**—WT and mutant protein solutions were prepared in a phosphate buffer (1 mg/ml) for CD studies using a J-720 spectropolarimeter (Jasco). The CD spectra were recorded in the far UV region (200–250 nm) using a 1-mm path length cuvette with a capacity of 200 ìl. The data obtained from the Jasco J-720 was transferred to Origo™ software (MicroCal Software, Inc.) for plotting and further data treatment. A series of experiments was conducted by observing the shifts of CD at various concentrations of CaCl2 for pH between 2.0 and 8.0 and for denaturation (Gdn-HCl) concentrations between 0 and 2 M. For pH-shifting experiments, the solution pH was adjusted to the desired value using 6 M HCl or 5 M NaOH. For additive Gdn-HCl experiments, the final solution was prepared by the dilution of 6 M Gdn-HCl stock solution with a protein solution to the desired concentration. Each experiment was completed by using at least 10–15 samples in different conditions.

**Protein Purification**—Protein solutions in a phosphate buffer (1 mg/ml) were prepared for fluorescence studies using a luminescence spectrometer LS50B (PerkinElmer Life Sciences). The fluorescence spectra were recorded at wavelengths between 300 and 550 nm and excitation at 298 nm using a 1-cm path length cuvette with a capacity of 700 ìl. The data obtained from the LS50B fluorometer were transferred to Origin software for plotting and further data treatment.

**Protein Measurement**—Protein solutions in a phosphate buffer (2 mg/ml) were prepared for thermal analysis using a Nano differential scanning calorimeter (Calorimetry Sciences Corp., Provo, UT). The results of DSC were recorded with the plot of heat capacity (Cp, kcal/ degree mol) versus temperature (15–70 °C). To remove bubbles, the sample solutions were degassed with sonication for 15 min before the thermal heating experiment. 1.5 ml of the sample was then loaded into the thermal chamber, and an equal amount of protein-free buffer was added as a reference. The pressure in the sample chamber was adjusted until 3 atmospheres was reached. The scan rate was 1 °C/min. The melting point (Tm) can be directly obtained from the DSC curve. The enthalpy change (ΔHf) was calculated by the integration of the curve-covering area using Origin software. A series of experiments under different environmental conditions such as the addition of denaturant, Gdn-HCl, were conducted, and the calculated ΔHf values were recorded.

**Crystallization of the E75G Protein**—Suitable crystals of the E75G protein were prepared as follows: a 20.5 mg/ml protein solution (10.5 mM potassium phosphate, pH 8.3) including a final concentration of 18% 2-methyl-2,4-pentadiol (w/w) was prepared. The mixture was incubated at 4 °C for 3 days. Afterward, 1 ml of 0.2 M equivalent of pdTp (2.5 mg/ml) and 2.0 M equivalent of calcium (200 mM CaCl2) were added, and the protein solution was filtered and crystallized against 40–50% 2-methyl-2,4-pentadial in 10.5 mM potassium phosphate at pH 8.3 using the hanging drop vapor diffusion method. Small, poorly diffracting crystals were formed at 4 °C within 3 months (19), whereas suitable single crystals (dimensions 0.2 × 0.2 × 0.1 mm) were obtained after 6 months. Data were collected on an X-ⅡⅡ
imaging plate detector using CuKα radiation generated by a RIGaku RU-300 rotating anode operating at 50 kV and 80 mA and processed using a commercial software package (Molecular Structure Corp.). The space group deduced from systematic absence is P4₁, and the unit cell parameters are as follows: a = b = 48.085 Å, c = 63.52 Å, which is isomorphous with wild type crystals. The resolution (Å), bond length (Å/angle (degrees) and dihedral (degrees) were 2.2, 0.012/1.849, and 25.626, respectively (the refinement statistics are not shown).

**Crystallographic Refinement**—Refinement of the E75G mutant crystal structure was carried out using the PROLSQ (20) and XPLOR (21) programs. The mutant model was derived from the wild type protein (22) using the CHAIN program (23). The Glu75 side chain was deleted from the initial model using the XPLOR program (21). After the initial model refinement, the maps were calculated with a resolution from 1.0 to 2.5 Å and 206 water molecules were located in the deletion maps. The solvent location, simulated annealing refinement, and PROLSQ based on standard B-DNA. The pdTp and calcium ion were restored on the maps, and 206 water molecules were located in the deletion maps. The solvent location, simulated annealing refinement, and PROLSQ refinement were processed and repeated three times to confirm system convergence. No additional solvent molecules could be located after refinement.

### Results

**Enzyme Kinetics**—Enzyme activity assays were done for WT SNase and the single point mutants E73G and D77G. The results are shown in Table I. The Michaelis constant ($K_m$) of the WT enzyme (2.63 ± 0.45 × 10⁻³) is 22 and 41% greater than E73G (2.05 ± 0.30 × 10⁻³) and D77G (1.54 ± 0.29 × 10⁻³), respectively. This implies that the dissociation rate of the enzyme-substrate complex is faster for the WT enzyme than for the mutants. The turnover rate ($k_{cat}$) representing the number of times each enzyme molecule catalyzes the reaction per unit time is greater for the WT enzyme (4.49 ± 0.80 × 10⁻³ s⁻¹) than for E73G (2.67 ± 0.51 × 10⁻³ s⁻¹) and D77G (2.20 ± 0.35 × 10⁻³ s⁻¹). The mutants also have lower apparent rate constants, $k_{cat}/K_m$, which represent the catalytic efficiency. The rate constant for E73G (1.30 × 10⁵ M⁻¹ s⁻¹) is 76% of the WT enzyme (1.71 × 10⁵ M⁻¹ s⁻¹), and the rate constant of D77G (1.43 × 10⁵ M⁻¹ s⁻¹) is 84% of the WT enzyme. The rate constant for the E75G mutant (24) is only 35% of the WT. The best fit of the nonlinear regression was used to calculate both $K_m$ and $k_{cat}$. Among the estimates, the smallest difference between E73G and D77G is still significant, since the t test values (25) (4 degrees of freedom) for the differences of $K_m$ and $k_{cat}$ (Table I) between E73G and D77G are 3.52 (>95% probability) and 2.85 (>95% probability), respectively. The t test values of the differences for the rest of the estimates have greater than 99% probability.

**Fluorescence and CD Spectra**—The intrinsic fluorescence of tryptophan residues can be used to detect shifts of a protein's tertiary structure or changes in tertiary structure caused by mutations. Fig. 2A shows the fluorescence emission curves of WT SNase and the E73G and D77G mutants in their native (N) (pH 7.0) and denatured (D) states (pH 2.5). A change in the maximum emission wavelength from N (340 nm) to D (355 nm) is observed for all three proteins. The intensity of fluorescence decreases significantly upon denaturation. This indicates that the Trp residues in the protein are significantly exposed to water upon unfolding. In the native state, the fluorescence intensities of the mutants are less than that of the WT protein, indicating that the exposure of Trp residues to water is greater for these mutants. Taken together, these data indicate that the confirmations of the mutated proteins are slightly relaxed compared with WT SNase.

Some typical CD spectra of WT SNase and the E73G and D77G mutants, in both N and D states, are shown in Fig. 2B. All proteins have similar secondary structures in their N states. In their D states, induced by the addition of 6 M Gdn-HCl, secondary structures are destroyed. Similar to the fluorescence data, these results show that the substitution of Gly for Glu at residue 73 or for Asp at residue 77 causes changes that primarily affect the protein's tertiary conformation rather than secondary structures.

**Protein Stability Measured by DSC**—The resistance of a protein to unfolding can be used to evaluate protein stability. The relative stability of the SNase mutants compared with the WT protein can be conveniently defined by thermal unfolding methods. Thermal analysis curves of WT SNase (solid line) and the E73G (dashed line) and D77G (dotted line) mutants are shown in Fig. 3A. A summary of the DSC results for these proteins, including $T_m$, $\Delta H_{m,cat}$ and percentage of stability, is presented in Table II. The calculated enthalpic changes ($\Delta H_{m,cat}$) are 84.1 ± 6.2, 65.6 ± 3.2, and 62.1 ± 2.5 kcal/mol for WT, E73G, and D77G, respectively. The E73G mutant has lost 22% of protein stability, and the D77G mutant has lost 26.2%. The $T_m$ values for WT, E73G, and D77G are 52.0 ± 0.6, 36.8 ± 0.2 and 43.9 ± 0.4 °C, respectively. The E75G mutant has the highest loss of stability (49.3%) compared with the WT protein. The E57G mutant retains the most stability, with only a 5.8% loss (19). E73G and D77G have intermediate stability losses of 22.1 and 26.2%, respectively. The thermal unfolding ($\Delta H_{m,cat}$) of the proteins as a function of Gdn-HCl concentration is shown in Fig. 3B. The midpoint transitions (the concentration of Gdn-HCl at half-maximum of $\Delta H_{m,cat}$ between the native and unfolded states of proteins) are 0.52, 0.27, and 0.31 M for WT, E73G, and D77G, respectively. Thus, the resistance to Gdn-HCl unfolding is greater for the WT protein than for the mutants.

The $t$ test value (5 degrees of freedom) for $\Delta H_{m,cat}$ (Table II) between E73G and D77G is 3.5 (>95% probability). For the $t$ test of the differences between mutant and WT proteins, the values are all greater than 95% probability except for E57G, which shows no significant difference in stability compared with the WT protein.

### Influence of Double/Triple Point Mutations on Protein Conformation and Stability

To further evaluate the effects of mutations of the charged residues Glu²⁷, Glu⁷⁵, and Asp⁷⁷ on protein conformation and on stability, we generated the double point mutants E73G/E75G, E75G/D77G, and E73G/D77G and the triple point mutant, E73G/E75G/D77G. Fig. 4A shows their fluorescence emission spectra. The relative fluorescence intensity is WT > E73G/D77G > E73G/D77G > E73G/E75G > E73G/E75G/D77G. Increases in the peak emission wavelengths are observed for the E73G/E75G and E73G/E75G/D77G mutants, which also demonstrate low fluorescence intensity. This
is especially true for the triple point mutant, where the fluorescence intensity is close to that of the denatured state (compare Fig. 4A with Fig. 2A). This indicates that the E73G/E75G and E73G/E75G/D77G mutants have very little tertiary conformation. A ladder of CD spectra is observed for these proteins (Fig. 4B). The strength of CD222 nm is \( \text{WT} / \text{H} \text{E73G/D77G} / \text{H} \text{E75G/D77G} / \text{H} \text{E73G/E75G} / \text{H} \text{E73G/E75G/D77G} \). The triple point mutant, E73G/E75G/D77G, has the lowest degree of secondary structure among the mutants. The pronounced change in secondary structure observed for the double/triple point mutants is not observed for the single point mutants (compare Fig. 4B with Fig. 2B).

Thermal analysis curves for the double and triple mutants are shown in Fig. 4C. The DSC curves for the double and triple mutants are shifted to lower temperatures relative to the DSC curve of the WT protein. Table III summarizes the thermal unfolding parameters for the mutant proteins. The E73G/E75G/D77G mutant has the lowest \( T_m \), 25.1 ± 0.5 °C. The \( \Delta H_{\text{cal}} \) values for protein unfolding are 84.2 ± 6.2, 45.0 ± 3.6, 34.3 ± 4.2, 35.6 ± 2.7, and 29.4 ± 1.9 kcal/mol for WT SNase and the E73G/D77G, E73G/E75G, E75G/D77G, and E73G/E75G/D77G mutants, respectively. The E73G/E75G/D77G mutant demonstrates a 65% loss of stability compared with the WT protein. The other three double point mutants have stability losses of 46–60%. The \( t \) test values (5 degrees of freedom) for \( \Delta H_{\text{cal}} \) (Table III) between mutants and WT are all better than 95% probability.

**X-ray Diffraction Analysis of the E75G Mutant**—The most sensitive position among single point mutations in the local area is at position 75 for Glu, which contributes to the loss in thermal stability of about 49.3%. The x-ray diffraction structure of the E75G mutant was therefore investigated. The final model structure of E75G consists of residues 7–141, Ca\(^{2+}\), pdTp, and 206 water molecules. The overall folding of the E75G mutant (Fig. 1) shows good geometry with r.m.s. deviations in bonds, angles, dihedral angles, and improper angles (data not shown) similar to those of wild type protein. The r.m.s. deviations for superimposition of C\(_\alpha\) atoms and for all equivalent atoms between the structures of mutant and wild type proteins are small (0.15 and 0.69 Å, respectively). The overall folding of the E75G mutant (Fig. 1) shows good geometry with r.m.s. deviations in bonds, angles, dihedral angles, and improper angles (data not shown) similar to those of wild type protein. The r.m.s. deviations for superimposition of C\(_\alpha\) atoms and for all equivalent atoms between the structures of mutant and wild type proteins are small (0.15 and 0.69 Å, respectively). The final R-factor and \( R_{\text{free}} \) are 0.173 and 19.3, respectively, for 7427 unique reflections between 10 and 2.2 Å (\( F_{\text{o}} > 3.0\sigma(F) \)). Ramachandran analysis of the E75G mutant indicates that both \( \phi \) and \( \psi \) angles of all residues occur in structure-allowed regions. The electron...
density map around residue 75 was calculated (Fig. 5) and indicated that the change around position 75 is unambiguous. In the WT, the side chain of Glu75 forms hydrogen bonds with Lys9, Tyr93, and His121 (Fig. 6A), whereas, in the mutant, a water molecule takes the position at the OE2 atom of Glu75 and is hydrogen-bonded with Tyr93 and His121, where the side chain of Lys9 no longer interacts with that water molecule and Gly75 (Fig. 6B).

**DISCUSSION**

The native conformation of SNase is maintained by noncovalent bonds in a state of dynamic equilibrium. The forces maintaining this flexible structure in solution are believed to be additive and correlated with one another. Mathematically, one cannot distinguish the relationships of these forces until the kinetic steps of protein folding/unfolding at the atomic level can be defined in detail. This task may not be possible at present because of the limitations of current instrumentation. At the molecular level, there is a variable contribution from each amino acid of a protein to the internal forces responsible for the protein’s structure and stability. Those amino acids that have the greatest contribution to these internal forces have a disproportionate influence on a protein’s overall structure. It is essential to identify these key regions if one is to determine the precise mechanism of protein refolding from a random unfolded state. To study the refolding of denatured SNase, Sinclair and Shortle used NMR techniques to identify the primary local interactions in the denatured state, which are predicted to be the most important interactions after the protein is collapsed to form its compact native conformation (26). These long range interactions among particular amino acids are considered "head start" groups that drive the change in conformation to the protein’s native state. The studies described in this paper complement the studies of long range interactions by characterizing the significant short range interaction forces. These involve the formation of stably local segments by amino acids that interact in the native state of SNase. These amino acids may drive key steps in the refolding of denatured SNase by either enhancing the rate of refolding or, because they interact preferentially, by inducing a particular route of refolding.

In studies of thermodynamically unfolded SNase, Baskakov and Bolen (27) determined the sizes of denatured ensembles of SNase proteins using fluorescence and size exclusion chromatography methods. Their results supported the notion that denatured ensembles of proteins exhibit "variable" thermody-
The results, which showed that the WT and single mutant unfoldings for these proteins, using a light-scattering method, were less compact than the native ones, since more amino acids from the hydrophobic core of the native proteins are exposed to water when they are unfolded. Our size distribution experiments have been altered. These mutants have considerably less contribution to the unfolding of the WT protein. When E75G is denatured by a chemical denaturant or change in pH, the C-terminal α-helix is unfolded through the I state (intermediate state) (30, 33), and the flexible β-barrel is subsequently destroyed.

In this report, we provide direct evidence that Glu75 plays an important role in the clamped area of SNase. In addition, the other two vicinal residues, Glu73 and Asp77, cooperate to maintain this locally stable structure. Evidence in support of this hypothesis can be inferred from the results of the enzyme activity assays for the single point mutants. The enzyme catalysis efficiency (kcat/Km) is reduced by about 65% for the E75G mutant but by only 24 and 16% for E73G and D77G mutants, respectively, relative to the WT protein (Table I). Similarly, the thermal enthalpic change of the E75G mutant is a 49% loss, while the E73G and D77G mutants exhibit a 22 and 26% loss, respectively, compared with the WT protein (Table II). Additional evidence for the importance of Glu75 is provided by the multipoint mutants. If Glu75 is present in the mutation, as is the case for E73G/E75G or E75G/D77G, the thermal stability loss is close to 60%. If not, as is the case for E73G/D77G, the thermal stability loss is ~47% (Table III). If all three positions are mutated, as for E73G/E75G/D77G, the loss in thermal stability increases to 65%.

In summary, an area of significant amino acid interactions has been identified, consisting chiefly of residues Glu75, Glu73, and Asp77, with other amino acids making smaller contributions. These forces are responsible for 65% of the structural stability of SNase.

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Significance of Local Electrostatic Interactions in Staphylococcal Nuclease Studied by Site-directed Mutagenesis

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