15N NMR relaxation studies have been used to characterize the backbone dynamics of the human acidic fibroblast growth factor (hFGF-1) in its free and sucrose octasulfate (SOS)-bound states. 15N longitudinal (R₁) and transverse (R₂) relaxation rates and (1H-15N) steady-state nuclear Overhauser effects were obtained at 500 and 600 MHz (at 25 °C) for all resolved backbone amide groups using 1H-detected two-dimensional NMR experiments. Relaxation data were fitted to the extended model free dynamics for each NH group. The overall correlation time (τₑ) for the free and SOS-bound forms were estimated to be 10.4 ± 1.07 and 11.1 ± 1.35 ns, respectively. Titration experiments with SOS reveals that the ligand binds specifically to the C-terminal domain of the protein in a 1:1 ratio. Binding of SOS to hFGF-1 is found to induce a subtle conformational change in the protein. Significant conformational exchange (R₂ex) is observed for several residues in the free form of the protein. However, in the SOS-bound form only three residues exhibit significant R₂ex values, suggesting that the dynamics on the micro- to millisecond time scale in the free form is coupled to the cis-trans-proline isomerization. hFGF-1 is a rigid molecule with an average generalized parameter (S²) value of 0.89 ± 0.03. Upon binding to SOS, there is a marked decrease in the overall flexibility (S² = 0.94 ± 0.02) of the hFGF-1 molecule. However, the segment comprising residues 103–111 shows increased flexibility in the presence of SOS. Significant correlation is found between residues that show high flexibility and the putative receptor binding sites on the protein.

Human acidic fibroblast growth factor (hFGF-1) is a 16-kDa protein, with a wide array of biological activities such as morphogenesis development, angiogenesis, and wound healing (1–4). The secondary structural elements in the protein include 12 β-strands arranged antiparallel into a β-barrel structure (5–7). hFGF-1 requires two kinds of receptors as follows: low affinity receptors that are heparan sulfate proteoglycans (HSPGs) and the high affinity receptors (FGFRs) that are transmembrane tyrosine kinases (2, 4, 8–10). The FGFRs are composed of an extracellular ligand binding portion consisting of three immunoglobulin-like domains (D1, D2, and D3), a transmembrane helix, and a cytoplasmic domain that contains the tyrosine kinase activity (11). The HSPGs are contemplated to bind and dimerize FGF and present the growth factor molecules to the receptor to form a ternary FGF-HSPG-FGFR complex (9, 11). The exact role of heparin/heparan sulfate in the activation of the hFGF-1 signaling pathway is still not clear. Some studies have shown that heparin binds to multiple FGF molecules and induces their oligomerization (8). Oligomerization of hFGF has been proposed as a prerequisite for binding and activation of the receptor (8). In contrast, results of other studies suggest a protective role for heparin/heparan sulfate (12–14). Binding of hFGF to the glycosaminoglycans is shown to protect FGFs against proteolytic digestion and heat- and acid-induced unfolding (15–17).

The high affinity tyrosine kinase activation and signaling are known to be dependent of FGF-induced dimerization. Dimerization of the receptor molecules is believed to lead to juxtaposition of the cytoplasmic domains and subsequent transphosphorylation on tyrosine residues in the cytoplasmic domain (11). Crystal structure of the FGF-FGFR complexed to its ligand (FGF) shows that the FGF-FGFR complex is partially stabilized by direct FGFR-FGFR interactions and by interactions between FGF and FGFR (11, 18). Interestingly, no direct FGF-FGF interactions were observed in the FGF-FGFR complex. The FGF-FGFR complex structure has a positively charged canyon and is believed to represent the heparin-binding site (11). However, to date the three-dimensional structure of the FGF-HSPG-FGFR ternary complex has not been solved.

Protein mobility plays an important role in the recognition process involved in protein-receptor, antigen-antibody, and enzyme-substrate interaction. One useful method to study motion of protein molecules involves the analysis of NMR relaxation processes (19, 20). With the advent of inverse detection methods, it has been feasible to analyze the backbone dynamics of proteins at a residue level using 15N relaxation measurements (21–26). In this background, we analyze the backbone dynamics of free hFGF-1 and its complex with a heparin structural analog sucrose octasulfate (SOS) using 1H-15N NMR techniques.

MATERIALS AND METHODS

Heparin-Sepharose was obtained from Amersham Pharmacia Biotech. Labeled 1HCl was purchased from Cambridge Isotope Laboratories. SOS was purchased from Toronto Research Chemicals. All other chemicals used were of high quality analytical grade. Unless mentioned, all solutions were made in 100 mM phosphate buffer (pH 7.0) containing 100 mM of ammonium sulfate. All experiments were performed at 25 °C.

Protein Expression and Purification—Residues are numbered as per their position in the primary structure of the 154 amino acid hFGF-1. Expression vector for the truncated form of the human FGF-1 (hFGF-1, residues 15–154) was constructed and inserted between the NdeI and BamHI restriction sites in pET20b. The plasmid containing the hFGF-1 insert was transformed into Escherichia coli BL21(DE3) pLysS. The expressed protein was purified on heparin-Sepharose using a NaCl gradient.
gradient (0–1.5 s). The protein was desalted by ultrafiltration using an Amicon set up. The homogeneity of the protein was assessed using SDS-polyacrylamide gel electrophoresis. The authenticity of the sample was further verified by electron spray-mass analysis. The concentration of the protein was estimated from the extinction coefficient value of the protein at 280 nm.

Preparation of Isotope Enriched hFGF-1—Uniform 15N isotope labeling was achieved using M9 minimal medium containing 15NH4Cl. In order to realize maximal expression yields, the composition of the M9 medium was modified by the addition of a mixture of vitamins. The expression host strain E. coli BL21(DE3)pLysS is a vitamin B1-deficient host, and hence the medium was supplemented with thiamine (vitamin B1). Protein expression yields were in the range of 25–30 mg/liter of the host, and hence the medium was supplemented with thiamine (vitamin B1). Protein expression yields were in the range of 25–30 mg/liter of the host, and hence the medium was supplemented with thiamine (vitamin B1). Protein expression yields were in the range of 25–30 mg/liter of the host, and hence the medium was supplemented with thiamine (vitamin B1).

15N Relaxation Measurements—15N longitudinal transverse relaxation and NOE data were collected on Varian Inova 500 MHz and Bruker DMX 600 MHz NMR spectrometers. Protein samples for relaxation measurements were dissolved at a concentration of ~1.5 mM in 100 mM phosphate buffer (pH 6.5) (containing 100 mM ammonium sulfate) prepared using 10% D2O and 90% H2O (at 25 °C). A 1:1 hFGF-1:B1). Protein expression yields were in the range of 25–30 mg/liter of the host, and hence the medium was supplemented with thiamine (vitamin B1). Protein expression yields were in the range of 25–30 mg/liter of the host, and hence the medium was supplemented with thiamine (vitamin B1).

The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium. The extent of 15N labeling was verified by isotope-enriched medium.

| Model | Spectral density function | Optimized parameters | No. of residues for free hFGF-1 | No. of residues for SOS-bound hFGF-1 |
|-------|---------------------------|----------------------|-------------------------------|----------------------------------|
| 1     | J(o) = 2/5[5(2T2)2(1 + (ωm,τ)2)] | S2, S2       | 22                           | 14                               |
| 2     | J(o) = 2/5[5(2T2)2(1 + (ωm,τ)2)] | S2, S2       | 38                           | 58                               |
| 3     | J(o) = 2/5[5(2T2)2(1 + (ωm,τ)2)] | S2, S2       | 8                            | 0                                |
| 4     | J(o) = 2/5[5(2T2)2(1 + (ωm,τ)2)] | S2, S2       | 12                           | 3                                |
| 5     | J(o) = 2/5[5(2T2)2(1 + (ωm,τ)2)] | S2, S2       | 13                           | 24                               |

\( \tau_e = \tau_m \tau_s / (\tau_m + \tau_s) \) is the overall rotational correlation time of the molecule. \( \tau_s = \tau_m \tau_p / (\tau_m + \tau_p) \) is the effective correlation time for internal motion on a fast time scale (\( \tau_s < 100 \) ps); \( \tau_e = \tau_m \tau_p / (\tau_m + \tau_p) \) is the effective correlation time for internal motion on a slow time scale (\( \tau_e < \tau_s < \tau_m \); \( S_2 = S_2^2/5 \)). \( S_2^2/5 \) is the generalized order parameter characterizing the amplitude of the internal motions, and \( S_2^2 \) and \( S_2^4 \) are the order parameters for the internal motions on the fast and slow time scales, respectively. The order parameters specify the degree of spatial restriction of the \( ^1 \)H-15N bond vector, with values ranging from zero for isotropic internal motions to unity for completely restricted motion and represent dynamics on the pico- to nanosecond time scale.

RESULTS AND DISCUSSION

hFGF-1 Ligand Interaction—SOS is a disaccharide structural analogue of heparin and has been shown to possess anticular properties (33). SOS has been shown to protect hFGF-1 from pH-induced and thermal-induced inactivation. SOS has been shown to bind to heparin-binding sites on hFGF-1 and stimulate the angiogenic and mitogenic activities of the protein (33). In vitro studies have revealed that unlike heparin, which oligomerizes the protein, SOS maintains the hFGF-1 molecule in a monomeric state (3). Thus, SOS effectively mimics the structural and functional properties of heparin and provides a scope to understand the effects of heparin on the backbone dynamics of hFGF-1 using \( ^15 \)N NMR relaxation methods.

The binding of SOS to hFGF-1 was followed by collecting a series of \( ^15 \)N HSQC spectra at varying protein to ligand ratios (1:0 to 1:1.5 ratio). All the cross-peaks in the spectra were assigned based on the previously published \( ^1 \)H and \( ^15 \)N NMR assignments of hFGF-1 (7). The \( ^1 \)H-\( ^15 \)N HSQC spectrum complexed to SOS at a 1:1 ratio shows that the chemical shift of...
rate parameters $R_1$, $R_2$, and NOE were obtained by analysis of dynamic stability of the protein.

It appears that binding of SOS to the residues at the C-terminal (residues 120–142) of the protein (6) shown to bind to a cluster of positively charged residues located in the C-terminal domain (residues 120–142) of the protein (6). This binding of SOS to protein is specific and the binding sites are restricted to definite regions(s) of the structure of hFGF-1. This observation is quite consistent with the crystal structure of the hFGF-1-SOS complex, whereas the ligand (SOS) has been shown to bind to a cluster of positively charged residues located in the C-terminal domain (residues 120–142) of the protein (6). It appears that binding of SOS to the residues at the C-terminal domain decreases the charge repulsion encountered by the cationic residues located at close proximity in this region (in the C-terminal domain) and consequently increases the thermodynamic stability of the protein.

**Effect of SOS on the Relaxation Parameters**—The relaxation rate parameters $R_1, R_2$, and NOE were obtained by analysis of proton-detected $^{15}$N and $^1$H heteronuclear correlation spectra of the free and SOS-bound form of hFGF-1. Reliable quantitation of peak intensities were possible for 110 out of the expected 140 $^{15}$NH backbone resonances in both the free and SOS-bound forms of hFGF-1. The residues for which the relaxation data could not be acquired include 7 prolines, or those whose resonances are highly overlapped (7 residues) or too weak to quantify accurately (16 residues). $^{15}$N relaxation data were collected at 25°C at 500 and 600 MHz field strengths. In all cases, the decay in intensity for all residues is found to be strictly exponential for both the $^{15}$N $R_1$ and $R_2$ data (at both 500 MHz and 600 MHz).

$R_1$ and $R_2$ values are sensitive to different motional frequencies. $R_1$ values provide information about motional properties with a frequency of approximately $10^7$–$10^8$ s$^{-1}$, whereas $R_2$ values, in addition to depending on motions occurring at these frequencies, are also sensitive to dynamics on the microsecond-millisecond time scale. Hence, by measuring both $R_1$ and $R_2$, it is feasible to obtain dynamic information over a large motional regime. The $R_1$ and $R_2$ values of hFGF-1 in its free and SOS-bound forms as a function of the residue number are shown in Fig. 3, panels A and B.

The $R_1$ values for the free form of hFGF-1 are remarkably uniform over most of the molecule with mean values of $1.17 \pm 0.07$ and $1.61 \pm 0.08$ s$^{-1}$ at 500 and 600 MHz, respectively. However, significantly greater than average $R_1$ values are seen for several residues in the free form of hFGF-1. 18 residues show an $R_1$ value greater than 1.25 s$^{-1}$. Three residues, namely Gly-76 ($1.38 \pm 0.13$ s$^{-1}$), Glu-118 ($1.33 \pm 0.06$ s$^{-1}$), and Ser-132 (1.43 ± 0.05 s$^{-1}$), exhibit extraordinarily large $R_1$ values (Fig. 3, panel A). Although the residues exhibiting noticeably higher $R_1$ values (>1.2 s$^{-1}$) are scattered throughout the hFGF-1 molecule, most of the residues located in the stretch spanning Tyr-78 to Leu-86 exhibit large $R_1$ values indicating a restricted mobility in this region of the protein molecule in its free state. It is interesting to note that 10 residues display $R_1$ values lower than the average $R_1$ values ($R_1 < 1.10$ s$^{-1}$). Some of these residues that show increased mobility are known to constitute the binding sites for heparin in the protein (Fig. 3, panel A). The average $R_1$ (at 600 MHz) of the protein molecule almost remains unchanged (average $R_1 = 1.17 \pm 0.07$ s$^{-1}$) upon complex formation with SOS, implying an overall similarity in the mobility of residues in the protein in the free and ligand-bound forms. Nine residues in the SOS complexed form reveal $R_1$ values significantly higher (>1.25 s$^{-1}$) than the trimmed average (Fig. 3, panel B). Most of the residues comprising the heparin-binding site show marginally increased $R_1$ values indicating a decrease in the nanosecond-time scale motion (of these residues) upon binding to SOS.

Although $R_1$ and $R_2$ are influenced by motions over a range of time scales, the transverse relaxation rate, $R_2$, is more sensitive to lower frequency (nanosecond) motions and also reflects contributions from slower millisecond or microsecond exchange processes that may cause line broadening in the NMR spectrum. The $R_2$ values for the free form of hFGF-1 are uniform for most of the molecules, with mean values of 13.07 ± 2.1 and 15.06 ± 2.9 s$^{-1}$ at 500 and 600 MHz, respectively. The $R_2$ values at 600 MHz are consistently higher than the values at 500 MHz. There are several residues in the free form of the protein that display $R_2$ relaxation values greater than the trimmed average (Fig. 3, panel A). Most notable among them are Gly-34 ($21.19 \pm 1.65$ s$^{-1}$), Glu-54 ($25.72 \pm 0.81$ s$^{-1}$), His-55 (20.27 ± 1.1 s$^{-1}$), His-107 (32.07 ± 0.38 s$^{-1}$), Lys-115 (18.41 ± 0.51 s$^{-1}$), Glu-118 (17.76 ± 0.37 s$^{-1}$), Trp-121 (20.02 ± 1.28 s$^{-1}$), and Leu-147 (19.14 ± 1.90 s$^{-1}$). Similarly, many residues at the N- and C-terminal ends in the free state of the protein show $R_2$ values significantly lower than the trimmed average (Fig. 3, panel A). In addition, the residues in the segments spanning Ala-62 to Gly-66, Leu-87 to Asn-94, and Tyr-108 to Ser-113 (with the exception of Asn-109) show $R_2$ values lower also than the trimmed average value (Fig. 3, panel A).

The average $R_2$ relaxation value of hFGF-1 is found to increase upon binding to SOS (average $R_2 = 17.37 \pm 3.16$ s$^{-1}$ at 600 MHz, Fig. 3, panel B). The general trends of the $R_2$ values in the free and ligand complexed states of the protein are similar. However, in all cases, the $R_2$ values of residues in the presence of SOS are higher than that observed in the ligand free state of the protein. In the SOS-complexed form, many residues in the C-terminal segment (Arg-136, Ala-143, Leu-135, and Leu-147) show $R_2$ values significantly greater than >19 s$^{-1}$, the trimmed average (Fig. 3, panel B). Interestingly, these residues in the free state of the protein possess $R_2$ values that are in the range of the average $R_2$ relaxation rate (in the free form of the protein).

In general, residues exhibiting low $R_2$ values are believed to
be involved in internal motions on the nano- and picosecond time scale. In this background, the residues at the N- and C-terminal ends that have low $R_2$ rates appear to undergo rapid, nanoseconds-picoseconds internal dynamics. It is interesting to note that there are residues distributed in three segments, namely Ala-62 to Gly-66, Leu-84 to Asn-94, and Tyr-108 to Ser-113 (with the exception of Asn-109) which are in rapid motions on the nano- or picosecond time scale. Some of the residues involved in these segments of the protein have been shown to represent the contact sites for the hFGF-1/receptor recognition (11, 18). However, a similar analysis of the residues exhibiting exceptionally large $R_2$ could not be provided because anomalously large $R_2$ rates could be due to either large contributions from conformational exchange dynamics ($R_{\text{ex}}$) on the micro- to millisecond time scales or due to anisotropic rotational diffusion of the protein (to be discussed later).

**{1H}-15N NOE Data**—{1H}-15N NOE data such as the relaxation of longitudinal and transverse magnetization of backbone $^{15}$N nuclei are sensitive to motions on a nanosecond to picosecond time scale. The $^{1H}$-$^{15}$N NOE is typically most sensitive to higher frequency motions of the backbone, with values near 1.0 indicating a lack of such motions, and lower values indicating increased local flexibility of the polypeptide (24). The average $^{1H}$-$^{15}$N steady-state NOE values estimated for free hFGF-1 at 600 and 500 MHz are $0.79 \pm 0.18$ and $0.73 \pm 0.21$, respectively. Most of the residues, which show low $R_2$ values (lower than the trimmed average), also depict substantially smaller NOE values ($<0.6$ at 600 MHz). Binding of the ligand increases the average NOE value (0.811 ± 0.19, at 600 MHz). This is in conformity with the trends in the $R_1$ and $R_2$ relaxation rates (Fig. 3, panels A and B). The protein appears to turn more rigid upon binding to the ligand. In general, the trends in the NOE values are consistent with those of the $R_1$ and $R_2$ measurements.

**Estimation of the Overall Correlation Time ($\tau_m$)**—The relaxation data in this study have been analyzed by using a model, which assumes isotropic motion. This assumption seems reasonable as the correlation time, $\tau_m$, calculated in the present study for each individual $^{15}$N nucleus from $T_1/T_2$ ratio are mostly similar throughout the protein backbone suggesting isotropic motion. Recent studies on the molecular dynamics of basic FGF (a structural homologue of hFGF-1) strongly suggest an isotropic rotation for the proteins belonging to the FGF family (34). Elegant backbone dynamics studies on interleukin
1β, which is a β-barrel protein and structurally homologous to hFGF-1, also indicate an isotropic tumbling for the β-barrel proteins (32). For the τm estimation of the protein (in the free and SOS-bound states), the residues that have a significantly smaller NOE (NOE < 0.6), are excluded because for these residues the assumption that motion on the τm (overall correlation time) time scale does not contribute to T1 relaxation is not valid.

In total, 9 residues in the free form and 6 residues in the SOS-bound state have NOE values lower than 0.6 (measured at 600 MHz). In addition, an increase in the R2 value could occur when conformational averaging on a time scale that is not extremely fast as compared with the difference in 15N chemical shifts. For a nearly spherical protein these residues could be identified by comparing the R2/R1 ratio observed for a given 15N nucleus with the average ratio, and by excluding residues that fall outside the S.D. However, this procedure is only valid for a protein with isotropic tumbling. The residues contributing to the anisotropic motion could be identified easily because any deviation from the average R2/R1 ratio, caused by motional anisotropy, is expected to affect both R1 and R2 in opposite ways. Therefore, residues undergoing conformational averaging would have larger R2 without a concomitant increase in R1. Hence, for calculation of the optimized τm, we excluded all residues wherein the R2 value exceeds the average R2 by one S.D. This procedure warranted the elimination of six residues for the R2/R1 optimization. The optimized τm for hFGF-1 in its free state was estimated to be 10.4 ± 1.0 ns. This value is consistent with the τm estimated for other proteins in the same molecular weight range. Moy et al. (34), as mentioned earlier, reported a τm value of 11.4 ns for basic FGF (−16 kDa) which is structurally homologous to hFGF-1. Estimation of the average τm value of hFGF-1 when bound to SOS did not significantly alter the overall correlation time (11.1 ± 1.3 ns). These results suggest that binding of SOS to hFGF-1 does not oligomerize the protein. In the event of ligand-induced dimerization/oligomerization, the τm value of the protein in the ligand-bound state is expected to be approximately twice the value for the protein in its monomeric form (23, 34). Comparison of the elution properties of hFGF-1 in its free and SOS-bound states also revealed that the protein (hFGF-1) exists in a monomeric state even after binding to the ligand (data not shown). In addition, our findings on the molecular state of the hFGF-1-SOS complex is consistent with the studies of Spivak Kroizman et al. (3), wherein FGF was shown to exist in a monomeric state upon binding to SOS.

Motions on the Microsecond-Millisecond Time Scale—In order to interpret the relaxation properties more qualitatively, the relaxation data were subjected to model free analysis. The models of the spectral density are similar to those used by Clore et al. (32). The information on the models of the spectral density that give the best fit to the experimental relaxation data are given in Table I. In general, for each backbone amide group, the dynamic model employing the fewest parameters, but capable of reproducing relaxation parameters, was identified.

Large values of R2 are generally indicative of line broadening due to chemical exchange (22). Assuming exchange between two states, these motions are typically on the milli- to microsecond time scale. A rigorous interpretation of the R2 is complicated as this parameter is a function of not only the rate constant for exchange but also the chemical shift difference and fractional populations at the exchanging sites (23). Hence, it could only be suggested that R2 term reflects the existence of dynamic exchange process in the milli- to microsecond time scale. In principle, the presence of the conformational exchange can be confirmed by R2 measurement at two different fields. An increase in the static magnetic field is expected to potentiate the chemical exchange contribution by increasing the difference in the resonance frequency of the 15N nucleus in the two environments (22). The contribution of R2 is proportional to the square of the 15N resonance frequency, for a two-state chemical exchange process (19). R2 terms determined at 500 MHz are predicted to be 70% of those determined at 600 MHz (22). In the free form of hFGF-1, 21 residues fit into this criteria and could be attributed to slow motions on the milli- to microsecond time scale (Fig. 4, panel A). The residues undergoing slow conformational exchange are spread throughout the protein sequence. Hence, it appears that the conformational exchange observed in hFGF-1 reflects slow global inter-conversions between folded conformations and not local order/disorder transitions.

The slow conformational motions observed in the free form of hFGF-1 could be possibly due to cis-trans-proline isomerization. There are seven proline residues in hFGF-1. Of these two proline residues are located at the N-terminal end, and the remaining prolines are distributed evenly in the three-dimensional structure of hFGF-1. Interestingly, except for Arg-102 most of the other residues showing high R2 values (Rex > 60 s−1) are located at close proximity to the proline residues. It is of interest to note that only three residues exhibit significant slow conformational motions in the SOS-bound state of the protein (Fig. 4, panel B). As already discussed, SOS binds to hFGF-1 in a 1:1 ratio at specific sites located in the C-terminal domain of the protein. It appears that binding of SOS to the protein thwarts the slow conformational motions (found in the free form) possibly associated with cis-trans-proline isomerization. Although SOS binds locally to the C-terminal domain, the binding of the ligand appears to have a global stabilizing effect on the protein conformation. This is consistent with the drastic increase observed in the global stability of the hFGF-1 upon binding to SOS (33). Moreover, the increase in average NOE value for the SOS-protein complex also supports the global effects of SOS on the conformation of hFGF-1. Additionally, it would be pertinent to mention that SOS is found to have significant effects on the cis-trans-proline isomerization process observed during the refolding of hFGF-1.2 In the presence of SOS, the refolding of hFGF-1 is significantly accelerated, and slow phase attributed to proline isomer-

2 Y.-h. Chi, T. K. S. Kumar, and C. Yu,, unpublished results.
ization was found to be missing. These findings probably indicate that binding of SOS to hFGF-1 inhibits the slow conformational motion observed in the milli- to microsecond time scale. However, more direct experimental evidence is needed to substantiate the correlation between cis-trans-proline isomerization and the observed $R_{\alpha}$ values in hFGF-1.

Estimation of the Generalized Order Parameter—Internal motions on the nanosecond to picosecond time scale result in the generalized order parameter ($S^2$) values that are less than unity. The average $S^2$ value of hFGF-1 in its free form is 0.89 ± 0.03 indicating a well ordered backbone structure with restricted internal motion on the nanosecond-picosecond time scale. In the free form, the putative heparin-binding site, comprising residues 120–142, shows an average $S^2$ value of 0.933 (Fig. 5, panel A). In addition to the N-terminal segment (residues 30–35), which exhibits an average $S^2$ value of 0.75 ± 0.04, there are several pockets in protein that exhibit rapid motions (0.8 < $S^2$ < 0.55) on the nanosecond-picosecond time scale. The most prominent among them are Asp-46 ($S^2$ = 0.73 ± 0.03), Gly-89 ($S^2$ = 0.48 ± 0.01), and Tyr-139 ($S^2$ = 0.76 ± 0.08). Most of the $\beta$-strands comprising the $\beta$-barrel structure exhibit average $S^2$ values in the range of 0.85–0.88. However, the average $S^2$ values of $\beta$-strands IX–XI are greater than 0.92. Interestingly, native state hydrogen-deuterium (H/D) exchange data on hFGF-1 also reveal that the residues in $\beta$-strands IX and X are the most protected from deuterium exchange.\(^6\)

The average $S^2$ (0.94 ± 0.02) value of hFGF-1 is found to increase upon binding to SOS. Thus, it appears that binding of SOS renders the hFGF-1 molecule more rigid by decreasing the overall internal motion. This feature is consistent with the conclusions drawn based on the $R_{\alpha}$, NOE, and $R_{\text{ex}}$ values. The average $S^2$ value of the heparin binding domain (residues 120–142) is also found to increase ($S^2$ = 0.96) in the presence of SOS. Although SOS decreases the overall flexibility of the hFGF-1 molecule, there are several residues that show increased internal motions when the protein is bound to SOS. For example, Lys-26 ($S^2$ = 0.67 ± 0.01), Lys-30 ($S^2$ = 0.76 ± 0.04), Arg-49 ($S^2$ = 0.82 ± 0.01), Ile-56 ($S^2$ = 0.54 ± 0.04), Glu-74 ($S^2$ = 0.79 ± 0.005), Ala-80 ($S^2$ = 0.68 ± 0.05), Leu-103 ($S^2$ = 0.80 ± 0.01), Glu-104 ($S^2$ = 0.69 ± 0.04), Tyr-108 ($S^2$ = 0.82 ± 0.01), and Tyr-111 ($S^2$ = 0.77 ± 0.01) exhibit an average $S^2$ value less than or equal to 0.82. Interestingly, all these residues exhibit $S^2$ values greater than 0.88 in the free form of the protein. Hence, it appears that SOS induces a subtle conformational change whereby residues that have lesser flexibility in the free state are released from their restricted environment (in the SOS-bound state). These minor conformational changes are not detected by optical techniques as the conformational change induced by SOS probably occurs at sites remote from the location of the optical probe(s) such as the tryptophan residue(s).

However, the postulated conformational change(s) is detected by the chemical shift perturbation plot shown in Fig. 2. Several residues outside the heparin-binding site (residues, 120–142) also experience a small change in their chemical shifts upon binding to SOS.

Comparison of the Backbone Dynamics of Interleukin 1β and hFGF-1—The three-dimensional structures of Interleukin 1β and hFGF-1 exhibit strong structural homology. Although both proteins share only 10–15% sequence similarity, their backbone in 9 of the 12 $\beta$-strands could be superimposed with a root mean square deviation of 0.5 Å (35). Comparison of the generalized order parameter ($S^2$) values in the free form of hFGF-1 and interleukin 1β shows striking similarities. Like in hFGF-1, most of the residues in interleukin 1β exhibit $S^2$ values greater than 0.8. There are three segments in the backbone of interleukin 1β that depict relatively higher flexibility ($S^2$ ~ 0.6). These segments correspond to unstructured loop regions connecting $\beta$-strands II and III, $\beta$-strands III and IV, and $\beta$-strands IV and V. Interestingly, residues in two of the corresponding loop regions connecting $\beta$-strands III and IV and $\beta$-strands IV and V in hFGF-1 also show higher flexibility ($S^2$ ~ 0.7). In addition, both in interleukin 1β and hFGF-1, many residues located in the $\beta$-strands IX–XI show relatively higher rigidity ($S^2$ > 0.9) than those situated in the other $\beta$-strands comprising the $\beta$-barrel structure. The similarities observed in the structure and the backbone dynamics of interleukin 1β and hFGF-Receptor could be the basis for the cytokine activity exhibited by these proteins.

FGF-Receptor Interaction Sites—Analysis of the backbone dynamics data also offers useful information on hFGF-1-receptor interactions. As free hFGF-1 has no or little biological activity, it appears that the protein complexed to SOS represents the biologically active state. It appears that the small conformational change induced by SOS is important for the FGF-receptor interaction. In this context, it is important to understand the internal motions of residues in the hFGF-1-SOS complex.

It is interesting to note that several residues such as Leu-103 ($S^2$ = 0.80 ± 0.01), Glu-104 ($S^2$ = 0.69 ± 0.04), Tyr-108 ($S^2$ = 0.82 ± 0.01), and Tyr-111 ($S^2$ = 0.77 ± 0.01) show relatively low $S^2$ values in the SOS-bound state of the protein. It appears that the marginally higher mobility of this segment (residues 103–111) is associated with the FGF-receptor recognition and binding. In this context, it important to mention that the segment spanning Arg-102 to Tyr-111 is believed to be intricately involved in the FGF-receptor interaction (10, 11, 18). Site-specific substitution of residues in this segment is known to result in a large decrease in the FGF-receptor binding affinity (10, 36, 37). Recent crystal structure of the FGF-receptor complex also reveals that residues in this segment are located at the FGF-receptor interface (11, 18). Similarly, the conformational flexibility of several residues at the N-terminal could also be rationalized in terms of the FGF-receptor interaction. The crystal structure of the FGF-receptor complex reveals that the N-terminal segment that is originally disordered in the free protein tends to become more ordered upon interaction with residues in the D3 domain of the receptor (11, 18).

We are unable to draw meaningful conclusions on the exceptionally low $S^2$ values exhibited by residues such as Ile-56 ($S^2$ = 0.54 ± 0.04) and Ala-80 ($S^2$ = 0.68 ± 0.05) based on the
existing literature on the FGF-receptor interactions. However, we are hopeful that future research on this subject would throw more light on the physiological relevance of the rapid internal motions displayed by these residues. Experiments based on native-state hydrogen-deuterium exchange are currently in progress to validate some of the findings reported in this study.

REFERENCES

1. Burgess, W. H., and Maciag, T. (1989) Annu. Rev. Biochem. 58, 575–606
2. Basile, C., and Mostacelli, D. (1992) Adv. Cancer Res. 59, 115–175
3. Spivak-Kozmin, T., Lemmon, M. A., Dikie, I., Ladbury, J. E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J., and Lax, I. (1994) Cell 79, 1015–1024
4. Pineda-Lucena, A., Jimenez, M. A., Lezane, R. M., Nieto, J. S., Santoro, J., Rico, M., and Gimenez-Gallego, G. (1994) J. Mol. Biol. 242, 81–89
5. Pineda-Lucena, A., Jimenez, M. A., Lezane, R. M., Nieto, J. S., Santoro, J., Rico, M., and Gimenez-Gallego, G. (1996) J. Mol. Biol. 264, 162–178
6. Zhu, X., Komiya, H., Chirino, A., Faham, S., Fox, G. M., Arakawa, T., Hsu, B., and Rees, D. C. (1991) Science 251, 90–93
7. Ogura, K., Nagata, K., Hatanaka, H., Habuchi, H., Kimata, K., Tati, S., Raveru, M. W., Jaye, M., Schlessinger, J., and Inagaki, F. (1999) J. Biol. Chem. 274, 16036–16047
8. Herr, A. B., Ornitz, D. M., Sasisekharan, R., Venkataraman, G., and Waksman, G. (1997) J. Biochem. (Tokyo) 272, 16382–16389
9. Faham, S., Linhardt, R. J., and Rees, D. C. (1998) Curr. Opin. Struct. Biol. 8, 578–586
10. Springer, B. A., Pantoliano, M. W., Barbera, F. A., Gunyuzhu, P. L., Thomp-son, L. D., Herbin, W. F., Rosenfield, S. A., and Boek, G. W. (1994) J. Biol. Chem. 269, 26879–26884
11. Ploitesnik, A., Schlessinger, J., Hubbard, S. R., and Mohammedi, M. (1999) Cell 98, 641–650
12. Mach, H., and Middaugh, C. R. (1994) Arch. Biochem. Biophys. 309, 36–42
13. Burke, C. J., Volkin, D. B., Mach, H., and Middaugh, C. R. (1993) Biochemistry 32, 6419–6426
14. Dabora, J. M., Sanyal, G., and Middaugh, C. R. (1991) J. Biol. Chem. 266, 23637–23640
15. Zhu, X., and Gallego, G. (1997) Eur. J. Biochem. 246, 328–335
16. Blaber, S. I., Culajay, J. F., Khurana, A., and Blaber, M. (1999) Biophys. J. 77, 470–477
17. Gospodarowicz, D., and Cheng, J. (1986) J. Cell. Physiol. 128, 475–484
18. Plotnikov, A., Hubbard, S. R., Schlessinger, J., and Mohammedi, M. (2000) Cell 101, 413–424
19. Palmer, A. G., III, Rance, M., and Wright, P. E. (1991) J. Am. Chem. Soc. 113, 4371–4380
20. Ye, J., Mayer, K. L., and Stone, M. J. (1999) J. Biomol. NMR 15, 115–124
21. Pascal, S. M., Yamaazaki, T., Singer, A. U., Kay, L. E., and Kay, J. D. (1995) Biochemistry 34, 11353–11362
22. Barbuto, G., Ikura, M., Kay, L. E., Pasto, R. W., and Bax, A. (1992) Biochem-istry 31, 5269–5278
23. Kay, L. E., Torchia, D. A., and Bax, A. (1989) Biochemistry 28, 8972–8979
24. Stone, M. J., Chandraashekhar, K., Holmgren, A., Wright, P. E., and Dyson, H. J. (1993) Biochemistry 32, 426–435
25. Stivers, J. T., Abeygunawardana, C., and Middaugh, A. S. (1996) Biochemistry 35, 16036–16047
26. Farrow, N. A., Muhandiram, R., Singer, A. U., Pascal, S. M., Kay, C. M., Gish, G., Shoelson, S. E., Pawson, T., Forman-Kay, J. D., and Kay, L. E. (1994) Biochemistry 33, 5984–6003
27. Press, R. H., Flannery, B. P., Teukolsky, S. A., and vHerling, W. T. (1992) Numerical Recipes in Fortran, 2nd Ed., pp. 48–67, Cambridge University Press, UK
28. Leatherbarrow, R. J. (1992) Graft, Version 3.0, Erithacus Softwares Ltd, Staines, UK
29. Mandal, A. M., Akke, M., and Palmer, A. G., III (1995) J. Mol. Biol. 246, 144–163
30. Lipari, G., and Szabo, A. (1982) J. Am. Chem. Soc. 104, 4546–4559
31. Clore, G. M., Driscoll, P. C., Wingfield, P. T., and Gronenborn, A. M. (1990) Biochemistry 29, 7387–7401
32. Clore, G. M., Driscoll, P. C., Wingfield, P. T., and Gronenborn, A. M. (1990) Biochemistry 29, 7387–7401
33. Volkin, D. B., Verticalii, A. M., Marfia, K. E., Burke, C. J., Mach, H., and Middaugh, C. R. (1993) Biochim. Biophys. Acta 1203, 18–26
34. Muy, F. J., Chanda, P. K., Cosma, S., Pisano, M. R., Urbano, C., Whelum, J., and Powers, R. (1998) Biochemistry 37, 1495–1504
35. Erickson, A. E., Coussens, L. S., Weaver, L. H., and Mathews, B. W. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3441–3445
36. Jaye, M., Schlessinger, J., and Dionne, C. A. (1992) Biochim. Biophys. Acta 1135, 185–189
37. Zhu, X., Anchin, J., Ramnarayan, K., Zheng, J., Kawai, T., Meng, S., and Wolff, M. E. (1997) Protein Eng. 10, 417–421