Conformation and Self-association of Human Recombinant Transforming Growth Factor-β3 in Aqueous Solutions*

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The transforming growth factors-β (TGF-β) are important regulatory peptides for cell growth and differentiation with therapeutic potential for wound healing. Among the several TGF-β isoforms TGF-β3 has a particularly low solubility at physiological pH and easily forms aggregates. A spectroscopic structural analysis of TGF-β3 in solution has thus been difficult. In this study, circular dichroism spectroscopy was used to determine the secondary structural elements of TGF-β3. In addition, the aggregation of TGF-β3 was investigated systematically as a function of pH and salt concentration using a rapid screening method. Sedimentation equilibrium and sedimentation velocity analysis revealed that TGF-β3 exists predominantly in two major forms: (i) monomers in solution at low pH and (ii) large precipitating aggregates at physiological pH. Under acidic conditions (pH < 3.8) the protein was not aggregated. At pH ~3.9, a monomer↔dimer equilibrium could be detected that transformed into larger aggregates at pH > 4.1. Aggregation was pronounced in the pH range of 4.3 < pH < 9.8 with the aggregation maximum between pH 6.5 and 8.5. The aggregation process was accompanied by a structural change of the protein. The CD spectra were characterized by an isodichroic point at 209.5 nm indicating a two-state equilibrium between TGF-β3 dissolved in solution and aggregated TGF-β3. Aggregated TGF-β3 showed a higher β-sheet content and lower β-turn and random coil contributions compared with monomeric TGF-β3. Both the solution structure and the aggregate structure of TGF-β3 were different from the crystal structure. This was in contrast to TGF-β2, which showed very similar crystal and solution structures. Under alkaline conditions (pH > 9.8) the turbidity disappeared and a further conformational change was induced. The pH dependence of the TGF-β3 conformation in solution in the range of 2.3 < pH < 11.0 was reversible. Aggregation of TGF-β3 was, furthermore, influenced by the presence of salt. For pH > 3.8 the addition of salt greatly enhanced the tendency to aggregate, even in the very basic domain. Under physiological conditions (pH 7.4, ionic strength 164 mm) TGF-β3 has almost the highest tendency to aggregate and will remain in solution only at nanomolar concentrations.

Transforming growth factors-β (TGF-β) are multifunctional cytokines used for cellular communication. They are called growth factors for historical reasons (1) but their main function is to control cell proliferation and differentiation (2, 3) and to stimulate the synthesis of extracellular matrix proteins (4). TGF-β plays a major role in the response of cells and tissues to injury (5, 6). Five isoforms of TGF-β are known; however, only three of them are expressed in mammalians. All isoforms show a highly homologous sequence (>70% of conserved residues). For a given isoform the homology between proteins from different species is >95%. The isoforms have similar biological activities but exhibit differences in potency depending on the target cell examined (7, 8). TGF-β is produced by virtually all cell types as inactive precursors (1, 9) and receptors for TGF-β are universally distributed throughout the body. The inactive precursor is first cleaved into a latent complex, which upon activation by acidification represents the regulation step of the signaling process of TGF-β (7, 10). The activation of latent TGF-β in vivo by osteoclasts during bone resorption may be linked to the acidification (pH < 3) of the osteoclast pericellular space (11, 12).

The in vivo activation seems, however, to be more often caused by proteolytic cleavage involving plasmin (13) or calpain (14). A further mechanism is the enzymatic deglycosylation of the mannose 6-phosphate of the latency associated peptide (15). In addition, protease-independent conformational changes of the latent complex following binding to thrombospondin (16, 17) also leads to release of active TGF-β in vivo.

TGF-β2 was the first isoform for which the crystal structure could be solved with x-ray crystallography (18–22). Next, the solution structure of TGF-β1 was determined by heteronuclear magnetic resonance spectroscopy (23) and was found to be very similar to the crystal structure of TGF-β2. The detailed comparison revealed only small differences (mainly in the β-turns) which, however, could play an important role in receptor binding and isoform recognition (24). The crystal structure of TGF-β3 was also solved recently (25). Compared with the TGF-β2 crystal no essential differences in the tertiary structure were observed. Minor deviations were detected in the N-terminal α-helix and in the β-sheet loop regions. The well established differences in the biological activity of TGF-β2 and TGF-β3 (25) seem to depend on differences in the surface side chains rather than the tertiary structure. Alternatively, it could be argued that despite the rather similar crystal structures, the two peptides assume different structures in solution. This problem was investigated here with CD spectroscopy.

The biologically active TGF-β3s are homodimers consisting of two identical chains connected via a single interchain disulfide bridge (C77–C77), the latter being exposed to solvent. Henry W. McKnight.

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erodimeric TGF-β_{1,3} and TGF-β_{2,3} are also known but rather unusual (26). Reduction of the active dimer results in the formation of inactive monomers. Activity is not recovered by simple reoxidation of the protein (27). The 8 other cysteine residues form 4 intrachain disulfide bridges, leading to a structural feature called “TGF-β knot.” The knot is almost inaccessible to solvent and stabilizes the monomer structure. The monomer exhibits an elongated nonglobular structure with dimensions of about 60 × 20 × 15 Å³. As there is only a single interchain disulfide bridge between two monomers, hydrophobic interactions between the interface areas are supposed to be of major importance in stabilizing the dimer (18–22). In addition, the dimer is further stabilized by a network of hydrogen bonds, including several water molecules, located at well-defined positions in the hydrophilic cavities surrounding the intersubunit disulfide bridge.

In contrast to TGF-β_{1} and TGF-β_{2}, TGF-β_{3} shows a strong tendency to aggregate at physiological pH, making spectroscopic measurements at pH 7.0 rather difficult. Inspection of the crystal structure of TGF-β_{3} reveals many hydrophobic residues on its surface, which could explain its low solubility. In view of the functional differences between TGF-β_{2} and TGF-β_{3} and also of the therapeutic potential of TGF-β_{3}, a detailed characterization of TGF-β_{3} solubility and aggregation is required.

In this study we have characterized the solution structure of TGF-β_{3} under a variety of conditions with CD spectroscopy. The experimental CD spectra were deconvoluted into its secondary structural elements and compared with the predictions derived from the TGF-β_{3} and TGF-β_{2} crystal structures. In addition, the details of the aggregation process and the structural changes accompanying aggregation were investigated. To this purpose, CD spectra were recorded as a function of pH, protein concentration, and salt concentration. Analytical ultracentrifugation measurements were performed to study the size of the aggregates while titrating TGF-β_{3} from acidic to basic conditions and vice versa. UV spectroscopy was used to monitor the turbidity of the TGF-β_{3} solutions as a result of protein aggregation at different pH and salt conditions.

MATERIALS AND METHODS

Protein Synthesis—Biologically active, recombinant human TGF-β_{3} was prepared at Novartis Ltd. (Basel) by refolding in vitro the monomeric, denatured protein overexpressed in Escherichia coli (31). TGF-β_{3} has the sequence (24): ALDTNYCFRN LEENCCVRPL YIDFRQDLGW KWWHEPKGY ANFCGSGCPY LRSADITHS VLGLYNTPV EASASFCVCP QDLEPLTIL VYGRFTPKVEQ LSNMVYKSC CK CS. The proper folding of the purified dimeric proteins was confirmed with reverse phase high pressure liquid chromatography and electrospray ionization mass spectrometry. The biological activity of the recombinant protein was found to be identical to that of natural TGF-β_{3} (29). The minimum content in TGF-β_{3} was about 90%, with 10% impurities due to related substances. The molecular weight as determined by mass spectrometry was very close to the theoretical value of 25,427 g/mol, due to related substances. The molecular weight as determined by mass spectrometry was very close to the theoretical value of 25,427 g/mol, due to related substances.

RESULTS

Spectrophotometric Titration of TGF-β_{3}—Acids (10 mM H_{3}PO_{4}, pH 2.3) and basic (10 mM Na_{2}PO_{4}, pH 11.7) stock solutions, each containing 100 μg/ml (3.94 μM) TGF-β_{3}, were mixed at different ratios. The pH of the mixture was determined, and the UV spectrum (250–350 nm) was recorded in a 1-cm cuvette. Fig. 1A compares the UV spectra of the two starting solutions. The absorption maximum shifts from 277.4 nm at pH 2.3 to 284.0 nm at pH 11.7. The pH-induced changes in UV absorption spectra were reversible, i.e., the reverse titration from basic pH to acidic pH returned the original low pH UV spectrum. In the pH range of 3.8 ≤ pH ≤ 10.2 TGF-β_{3} was found to aggregate, causing an increase in UV absorbance due to light scattering. This is demonstrated in Fig. 1B for pH values of 4.8, 6.3, and 10.0. The protein content in all samples was 100 μg/ml (3.94 μM). Compared with the pH 2.3 spectrum (Fig. 1A), the shape of all spectra is distorted. Although TGF-β_{3} in solution does not show any absorption at λ = 350 nm, the aggregation of the peptide in the range of 4.1 < pH < 10.2 generates intensity at this wavelength due to light scattering. The light scattering is even more pronounced at a shorter wavelength, because the light scattering intensity increases with decreasing wavelength.

The TGF-β_{3} dimer contains 16 tyrosine residues. Upon ionization the absorption maximum of Tyr shifts from λ = 274.8 to 293.2 nm, with the corresponding extinction coefficients increasing from ε_{274.8} = 1,405 M⁻¹ cm⁻¹ to ε_{293.2} = 2,381 M⁻¹ cm⁻¹ (32). Difference UV spectra of TGF-β_{3} (3.94 μM) recorded at pH 2.3 and 11.7 yield a change in optical density at 295 nm of 0.08 for a 1 cm quartz cell. The concentration of deprotonated...
Tyrosine residues at pH 11.7 is thus approximately 33.6 μM, whereas the total concentration of tyrosine residues is 16 × 3.94 = 63 μM. The titration experiment therefore provides evidence that only about 50% of the Tyr residues are available for deprotonation at basic pH.

Aggregation of TGF-β3 as a Function of pH and NaCl Concentration—The effect of NaCl on TGF-β3 aggregation was studied with a microplate reader. 10 mM H₃PO₄ (pH 2.3) and 10 mM Na₃PO₄ (pH 11.7) stock solutions containing 64 μg/ml (2.5 μM) TGF-β3 were mixed at different ratios. Aggregation of TGF-β3 took place at pH > 3.8 and pH < 10.2 with the aggregation maximum around the physiological pH value. The increased absorbance at λ = 350 nm is caused by light scattering and is a quantitative measure of protein aggregation.

The effect of NaCl on TGF-β3 aggregation was also investigated. The solubility of TGF-β3 was found to be similar in those solvents/buffers as in phosphate buffer.

TGF-β3 Aggregation As a Function of Protein Concentration—The aggregation of TGF-β3 was further investigated as a function of the protein concentration in phosphate buffer (10 mM H₃PO₄, 10 mM Na₃PO₄). At a low pH value (<3.8) TGF-β3 could easily be dissolved up to concentrations of 150 μg/ml. No absorbance at 350 nm was observed. At approximately neutral pH values (5.7 ≤ pH ≤ 8.6), TGF-β3 was found to strongly aggregate. The turbidity at λ₃₅₀ increased linearly with increasing protein concentration. Even at a concentration as low as 5 μg/ml (~0.2 μM), TGF-β3 precipitation was noted.

The TGF-β3 self-association was studied in more detail at pH 9.7, with protein concentrations between 43.3 μg/ml (1.7 μM) and 168 μg/ml (6.6 μM). The absorbance at λ = 350 nm increased with the protein concentration. A plot of the optical density versus the protein concentration yielded a straight line intersecting the abscissa at 28 μg/ml (1.1 μM). Below this concentration no aggregation occurred at pH 9.7. In a second type of experiment the protein solutions (pH 9.7) were centrifuged for 30 min at 10,000 rpm in an Eppendorf centrifuge leading to a precipitation of aggregated TGF-β3. The protein concentration in the supernatant was determined with UV spectroscopy and was found to be constant with 28 μg/ml in all samples, in agreement with the turbidity measurements. At higher pH values (pH > 10.8, no NaCl), no aggregation was observed even at high protein concentrations (data not shown).

The effect of NaCl on TGF-β3 aggregation at different protein concentrations was also studied. 100 μl of an NaCl stock solution (400 mM) was added to 200 μl of TGF-β3 solution to study TGF-β3 self-association under physiological NaCl conditions (133 mM). Under acidic conditions (pH < 3.6) no increase in turbidity was observed; however, at higher pH values the addition of NaCl considerably decreased the protein solubility. Aggregation was observed at protein concentrations as low as 3.5 μg/ml, i.e., at approximately 10 times lower concentrations than in the absence of salt.

**Fig. 1.** UV spectra of TGF-β3 as a function of pH. A. UV spectra of TGF-β3 under acidic (100 μg/ml (3.94 μM) TGF-β3 in 10 mM H₃PO₄, pH 2.3) and basic conditions (100 μg/ml (3.94 μM) TGF-β3 in 10 mM Na₃PO₄, pH 11.7). The maximum absorption wavelength shifts from 277.4 nm at pH 2.3 to 284.0 nm at pH 11.7. No absorption is observed at 350 nm. B. UV spectra of TGF-β3 (c = 3.94 μM) near physiological pH. The protein solutions were made by mixing acidic (pH 2.3) and basic (pH 11.7) stock solutions at different ratios. Aggregation of TGF-β3 was further investigated as a function of the protein concentration in phosphate buffer (10 mM H₃PO₄, 10 mM Na₃PO₄). At a low pH value (<3.8) TGF-β3 could easily be dissolved up to concentrations of 150 μg/ml. No absorbance at 350 nm was observed. At approximately neutral pH values (5.7 ≤ pH ≤ 8.6), TGF-β3 was found to strongly aggregate. The turbidity at λ₃₅₀ increased linearly with increasing protein concentration. Even at a concentration as low as 5 μg/ml (~0.2 μM), TGF-β3 precipitation was noted.

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**Fig. 2.** Dependence of the TGF-β3 aggregation on pH and NaCl concentration monitored with UV spectroscopy at 350 nm. All measurements were performed with 65 μg/ml (2.56 μM) TGF-β3 in 10 mM H₃PO₄, 10 mM Na₃PO₄. At a low pH value (<3.8) TGF-β3 could easily be dissolved up to concentrations of 150 μg/ml. No absorbance at 350 nm was observed. At approximately neutral pH values (5.7 ≤ pH ≤ 8.6), TGF-β3 was found to strongly aggregate. The turbidity at λ₃₅₀ increased linearly with increasing protein concentration. Even at a concentration as low as 5 μg/ml (~0.2 μM), TGF-β3 precipitation was noted. The effect of NaCl on TGF-β3 aggregation at different protein concentrations was also studied. 100 μl of an NaCl stock solution (400 mM) was added to 200 μl of TGF-β3 solution to study TGF-β3 self-association under physiological NaCl conditions (133 mM). Under acidic conditions (pH < 3.6) no increase in turbidity was observed; however, at higher pH values the addition of NaCl considerably decreased the protein solubility. Aggregation was observed at protein concentrations as low as 3.5 μg/ml, i.e., at approximately 10 times lower concentrations than in the absence of salt.
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Table I

Sedimentation measurements of TGF-β3

| pH   | Sedimentation Velocity | Sedimentation Equilibrium |
|------|------------------------|---------------------------|
|      | $S_{20,W} (S)^a$ rpm | $M_r, kDa$ rpm           |
| 2.3  | 2.4 52,000            | 26.1 14,000              |
| 3.9  | 2.3 56,000            | 32.6 24,000              |
| 4.1  | 2.2: ~25% and 100b, ~75% 56,000 24,000 |
| 4.3  | 400 5,000             |                           |
| 7.6  | ~c 2,000              |                           |
| 9.7  | ~c 10,000             |                           |

State of Aggregation

- Monomer (25.4 kDa)
- ~70% monomers +30% dimers
- ~25% monomers + ~75% large aggregates
- Very large aggregates
- Large aggregates
- Large aggregates, aggregates at TGF-β3 > 30 μg/ml

Notes:

- Sedimentation coefficient corrected to the value it would have in a solvent with the viscosity and density of water at 20 °C (30).
- Broad and dispersed boundary.
- Protein aggregates are too large to be studied with analytical ultracentrifugation, even at low speed.
- Measured with UV spectroscopy.

Analytical Ultracentrifugation—The aggregation equilibrium in the pH range of 2.3 $\leq \text{pH} \leq 4.2$ was further analyzed with analytical ultracentrifugation. The protein concentration was 117.1 μg/ml (6.4 μM) in 10 mM phosphate buffer. At pH 2.3, the TGF-β3 solutions were investigated in a sedimentation equilibrium for 16 h at 24,000 rpm. Analysis of the equilibrium profile with a single component model yielded a molecular mass of 26.1 kDa, which is in agreement with the theoretical molecular weight of the TGF-β3 monomer (25.4 kDa).

Corresponding measurements were also performed at pH 3.9 yielding an apparent molecular weight of 32.6 kDa, indicating a monomer (~70%) ε dimer (~30%) equilibrium.

At pH 4.1, samples were centrifuged at rotor velocities of 24,000 rpm for 45 min. A diffuse boundary was observed containing about 75% of the total protein. TGF-β3 was found to associate in large nonspecific aggregates. Next, the samples were centrifuged for 3 h at 56,000 rpm. A second boundary was observed representing about 25% of the total protein and corresponding to the monomeric protein. At pH 4.1, TGF-β3 thus exists in two forms only, namely monomers (~25%) and large aggregates (~75%).

At pH 4.3 a broad, dispersed boundary was measured that, at 5,000 rpm, yielded only one phase with a high sedimentation coefficient. TGF-β3 was completely aggregated and formed large nonspecific aggregates.

Sedimentation studies at physiological pH 7.4 failed. Even at a low speed of 2,000 rpm no equilibrium conditions were reached. TGF-β3 was found to associate into large aggregates.

Sedimentation studies were also performed at basic pH. Low concentration samples of TGF-β3 (40 μg/ml) at pH 9.7 were centrifuged at 10,000 rpm, measuring the change in protein concentration at the limit of the UV detection system. Even under this low concentration conditions no sedimentation equilibrium was reached with the mass of TGF-β3 aggregates being still too large to be measured. This result is consistent with the turbidity measurements discussed above (Fig. 2) indicating TGF-β3 aggregation at protein concentrations $\leq 30 \mu g/ml$ at pH 9.7. The results obtained with analytical ultracentrifugation are summarized in Table I.

Conformational Studies of TGF-β3—The variation of the UV spectra with pH (Fig. 1A) suggests a conformational change of TGF-β3. To obtain insight into the molecular details of this process, CD spectra were measured in the pH range of 3.2 $\leq \text{pH} \leq 4.4$ and at pH 9.8 and are shown in the Fig. 3. In the intermediate pH range, the protein solutions were turbid and allowed only an approximate interpretation of the spectra. Inspection of Fig. 3 reveals an isodichroic point at $\lambda = 209.5$ nm, providing evidence for a two-state conformational equilibrium at low pH. The pH-induced conformational change was found to be reversible. The CD spectra at acidic pH are characteristic of essentially a β-sheet structure with additional contributions from random coil, β-turn, and α-helix (see below). The spectra at very basic pH are characterized by a distinct minimum around 217 nm and a positive ellipticity below 200 nm, indicative of an increased contribution of β-sheet conformation at high pH values.

The aggregation of the protein in the pH range 4.4 $\leq \text{pH} \leq 9.8$ leads to CD spectra that are distorted by light scattering. However, the observation of an isodichroic point provides evidence that the CD spectra, as far as measurable, have a shape intermediate between those shown in Fig. 3 for pH 4.4 and pH 9.8.

Discussion

CD and UV spectroscopy as well as ultracentrifugation studies demonstrate that TGF-β3 is soluble in monomeric form at pH $\leq 3.8$ and pH $\geq 9.7$. In contrast, the peptide aggregates at intermediate pH values with the aggregation maximum occurring at 6.8 $\leq \text{pH} \leq 8.2$ (cf. Fig. 2). Based on the amino acid sequence (24) and on the known pK values of amino acids free in solution (neglecting pK shifts induced by intrachain interactions), it is possible to calculate the net charge of TGF-β3 as a function of pH using the Henderson-Hasselbach equation. An
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FIG. 4. Comparison of experimental and simulated CD spectra of TGF-β3. Dotted line, simulated CD spectrum based on the TGF-β2/β3 crystal structure, pH ~ 4.5; solid line, experimental CD spectrum of TGF-β3 in 10 mM H3PO4, 10 mM Na3PO4, pH 4.4. The experimental spectrum is distorted and shifted due to light scattering and optical flattening of the slightly turbid solution. ●, simulation of the TGF-β3 CD spectrum with a secondary structure of 4% α-helix, 66% β-sheet, 8% β-turns, and 22% random coil.

The isoelectric point is estimated for pH = pI ~ 6.8. The solubility of TGF-β3 at low and high pH values could thus be explained by its large positive or negative electric charge, respectively, at these pH extremes. In contrast, aggregation at physiological pH could be induced by the rather hydrophobic surface of the electrically neutral protein. Analogous calculations for TGF-β1 and TGF-β2 yield isoelectric points of pI ~ 9.5 and pI ~ 8.5, respectively. In fact, TGF-β1 and TGF-β2 are distinctly more soluble than TGF-β3 under physiological conditions. The good solubility of TGF-β3 at low pH could explain its prominent role in vivo in processes involving acidification of the surroundings. The most striking examples are: (i) bone remodeling with pH < 3.0 around the osteoclasts (34–37); (ii) inflammation where lysosomal release can locally lower the pH under 5.0 (38–40); and (iii) the ubiquitous expression of TGF-β3 in gastric tissues and its strong influence in gastric cancers, in contrast to TGF-β1, which is localized principally in parietal cells, and TGF-β2, which is present exclusively in chief cells (41).

TGF-β2 was the first TGF-β isoform to be crystallized (at pH 4.5). The x-ray analysis yielded a structure with 11% α-helix, 36% β-sheet, 8% helical turns, 3% 3_10-helical turn and 42% random coil (22). In 1996, the crystal structure of TGF-β3 was also solved (25). Comparison with TGF-β2 revealed, however, only small differences, mainly in the β-loop regions (25). Because the percentages of structural elements were not specified by Mittl et al. (1996), TGF-β2 was taken as the starting point for the simulation of the CD spectra. Based on reference CD spectra of 18 globular proteins (31) and using the percentages of the crystal structure, the CD spectrum shown by the dotted line in Fig. 4 was calculated. The theoretical spectrum is quite different from the experimental spectrum (solid line), indicating that the TGF-β2/TGF-β3 crystal structure is not a good model for TGF-β3 in solution. A much better fit to the experimental spectrum at pH 4.4 is given by a simulation containing 4% α-helix, 66% β-sheet, 8% β-turns, and 22% random coil (● in Fig. 4). Compared with the crystal structure, the α-helical content is reduced and the contribution of β-structures is clearly enhanced. The displacement in the wavelength of the two spectra is due to spectral distortions caused by light scattering of the TGF-β3 aggregates.

The simulation of CD spectra is a multiparameter fit, and the relevance of the structural parameters is often subject to criticism. However, CD simulations are biased in sensitivity toward α-helical structures. The decrease of α-helix of TGF-β3 in solution compared with the crystal structure is unambiguous and clearly beyond the error of the numerical approach. A possible explanation for the reduced α-helical content of TGF-β3 in solution is the presence of glycine (Gly-63) in the α-helical region of TGF-β3 between amino acids 57 and 68. This glycine, which confers to the protein backbone additional flexibility, is not present in the α-helical regions of TGF-β1 and TGF-β2 (25).

CD spectra of TGF-β3 in solution at pH 1.9 have been reported previously, indicating a much larger helix content (28). We have not been able to confirm these results, which were probably caused by excessive smoothing of the spectra. The present findings are, however, in agreement with 2D-NMR studies of TGF-β3, which suggest an increased molecular flexibility in comparison with TGF-β1.2 The considerable structural difference between TGF-β3 in solution and the crystal structure suggests a rather flexible conformation that can adjust itself to external constraints. It could explain the different receptor specificity of TGF-β2 and TGF-β3 despite similar x-ray structures.

We have also recorded CD spectra of TGF-β2 in solution at pH 2.96 (data not shown) and have compared them with theoretical spectra calculated on the basis of the crystal structure. In contrast to TGF-β3, a good agreement between the experimental and theoretical spectral shapes was found for TGF-β2. The CD results for TGF-β2 may serve as a positive control for the sensitivity of CD spectroscopy to detect conformational changes for the problem at hand. They emphasize that TGF-β2

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adopts the same structure in the crystal and in solution, whereas TGF-β3 reveals two different conformations.

The CD spectra of TGF-β3 further demonstrate that the conformation of this protein varies with the pH of the solution. A first conformational change occurs at pH ~ 4.4 and is accompanied by TGF-β3 aggregation; the second transition begins at pH ~ 9.8 and is associated with the deprotonation of the solvent-accessible tyrosine residues (8 of 16; see above). At the same time the aggregation process is reversed. The pH-induced aggregation can also be detected with fluorescence spectroscopy using the TGF-β3 Trp residues as intrinsic markers. Aggregation leads to an increase in the steady state polarization and an increase of the fluorescence life time.3 A deconvolution of the CD spectra was attempted in the pH ranges of 2.2 ≤ pH ≤ 6.0 and 9 ≤ pH ≤ 10.4 where aggregation was not too pronounced. The relative contributions of the different secondary structures are summarized in Fig. 5. The most prominent change is the increase in β-structure around pH 4.4, which is reversed at pH ~ 9.8. The destabilization of the β-structure at pH ~ 9.8 is compensated by an increase in α-helix.

Concluding Remarks—In conclusion, the solubility of TGF-β3 under physiological conditions (pH 7.0) is low and distinctly smaller than that of TGF-β1 and TGF-β2. TGF-β3 has a high tendency to adsorb to hydrophobic surfaces and to form large aggregates. At extreme pH values (pH < 2.3 or pH > 11.3) TGF-β3 is monomeric in solution, whereas at pH ~ 3.9 a monomer ↔ dimer equilibrium was detected by ultracentrifugation. The addition of salt greatly reduces the solubility of TGF-β3 and enhances its tendency to aggregate. The structure of TGF-β3 in solution is different from the crystal structure; notably, the helix content is reduced, and the β-structure content is increased. The change in conformation of TGF-β3 in solution could explain the different receptor specificity of TGF-β3 compared with TGF-β2 despite their very similar x-ray structure.

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