Structural Analysis of Bovine Somatotropin Using Monoclonal Antibodies and the Conformation-Sensitive Immunoassay*

William P. Pfund‡, James S. Bourdage, and Kathleen A. Farley
From Analytical Research & Specification Development, Pharmacia & Upjohn, Inc., Kalamazoo, Michigan 49001

Bovine somatotropin was studied with respect to thermal stability, quantitative thermal denaturation kinetics, and refolding potential following thermal denaturation using a panel of 6 monoclonal antibodies and the Conformation-Sensitive Immunoassay (CSI). The antibody panel consisted of 4 conformation-dependent and 2 sequence-specific antibodies. Each of the antibodies revealed unique thermal stability profiles for their respective epitopes suggesting that they each recognize different antigenic determinants. Comparing the thermal stability profiles generated with these antibodies allowed the stability of bovine somatotropin to be "dissected" based on individual structural features. The degree to which bovine somatotropin is stabilized by disulfide bonds was examined using CSI-based quantitative thermal denaturation kinetics profiles generated under reducing and nonreducing conditions. All of the conformational epitopes unfolded faster under reducing conditions indicating that the two disulfide bonds within the somatotropin molecule impart some degree of global stabilization. The ability of bovine somatotropin to refold after reducing or nonreducing thermal denaturation was also examined using the antibody panel and the CSI. The results show that, although significant refolding was evident for some epitopes, bovine somatotropin cannot refold to the native state following thermal denaturation under either reducing or nonreducing conditions.

Conformation-sensitive monoclonal antibodies are becoming popular tools for investigating protein structure. Such antibodies are specific for defined conformational features and therefore allow structural studies to be focused on relatively small, defined areas of an antigen molecule. The specificity and sensitivity of conformation-sensitive monoclonal antibody probes has been demonstrated repeatedly. For example, the ability of conformation-specific antibodies to detect nearly native conformational features during refolding studies at times before spectroscopic probes are able to detect native structures allowed the stability of bovine somatotropin to be "dissected" based on individual structural features. The degree to which bovine somatotropin is stabilized by disulfide bonds was examined using CSI-based quantitative thermal denaturation kinetics profiles generated under reducing and nonreducing conditions. All of the conformational epitopes unfolded faster under reducing conditions indicating that the two disulfide bonds within the somatotropin molecule impart some degree of global stabilization. The ability of bovine somatotropin to refold after reducing or nonreducing thermal denaturation was also examined using the antibody panel and the CSI. The results show that, although significant refolding was evident for some epitopes, bovine somatotropin cannot refold to the native state following thermal denaturation under either reducing or nonreducing conditions.

EXPERIMENTAL PROCEDURES

Bovine Somatotropin—Bovine somatotropin was obtained from Dr. A. F. Parlow of the University of California at Los Angeles. The protein was solubilized in 30 mM NaHCO₃, 150 mM NaCl (pH 10.8) to yield a 10 mg/ml solution and then diluted to working concentrations (see text) in PBS (10 mM KPO₄ buffer, 150 mM NaCl, pH 7.3).

Monoclonal Antibodies—Production and preliminary characterization of the six bSt-specific monoclonal antibodies (mAbs) have been described previously (16). Qualitative CSI analyses of these antibodies has shown that Anti-bSt mAbs 1–3 recognize only native bSt while Anti-bSt-4 is capable of binding native and denatured bSt. Anti-bSt-7 is able to recognize native bSt and bSt denatured under nonreducing conditions, but fails to bind to bSt denatured under reducing conditions. Anti-bSt-16 was shown to exhibit significant reactivity with denatured bSt only.

Quantitative Conformation-Sensitive Immunoassay (CSI)—Analysis of bSt was performed as outlined in Fig. 1 using a modification of the CSI procedure previously reported (16). Immobilon™-AV affinity membranes (IAV), with a pore size of 0.65 μm, were obtained from Millipore.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Pharmacia & Upjohn, Inc., 7000 Portage Rd., Kalamazoo, MI 49002. Tel.: 616-833-3659; Fax: 616-833-6743; E-mail: WPPFUND@pwinet.Upj.Com.

1 The abbreviations used are: bSt, bovine somatotropin; mAb, monoclonal antibody; CSI, Conformation-Sensitive Immunoassay; IAV, Immobilon™-AV affinity membrane; PBS, phosphate buffered saline.
The membranes were prewet in PBS, and bSt samples (40-14056) were spotted onto membranes using a Bio-Dot™ vacuum filtration manifold (Bio-Rad). The membranes were then incubated at 25°C for 16–18 h between two sheets of blotting paper prewet in PBS to maximize the amount of protein in covalent complexes with IAV. Unbound IAV reactive sites were capped with 10% monoethanolamine in 1M NaHCO3 for valently binding the antigen to IAV and capping the membrane reactive sites to examine antigen refolding following thermal denaturation (4). The denaturation of antibody are shown in Table I, and typical standard curves reflecting densitometry, and results were normalized with respect to a standard curve of varying bSt concentrations processed under irreversible reducing conditions at 100°C for 5 min (100% free SH).

Optimization—Samples for NMR analysis were prepared by dissolving bSt as described above and diluting in PBS to obtain a solution with a concentration of 1 mg/ml and a pH of 9.7. The solution was then lyophilized to a dry cake and dissolved in D2O (Isotec 99.96% D) to a final protein concentration of 2 mg/ml and was adjusted with 1M DCl to a final pD of 7.4. The pH measurement was not corrected for possible isotope effects on the glass electrode.

The 1H spectra were recorded on a Bruker AMX-500 operating at 500.13 MHz using an inverse 5-mm Bruker triple resonance z-axis gradient probe. Each spectrum was collected using 600 μl of the protein solution, 64 transients, 16K data points, a spectral width of 6 kHz, an acquisition time of 1.3 s, and a HDO presaturation delay of 2 s. An exponential multiplication of 2 Hz was used prior to Fourier transformation. Chemical shifts are expressed relative to the internal standard, sodium-3-trimethylsilyl propionate (2,2,3,3-d4 (0.0 ppm)).

RESULTS

Assay Optimization—Optimization of the original (qualitative) CSI method was accomplished for each antibody by establishing a fixed concentration of antibody (0.5 μg/ml) and determining antigen concentrations that allowed for a linear relationship between antigen concentration and spot density. The antigen concentrations yielding linear response for each antibody are shown in Table I, and typical standard curves.
prepared for two mAbs are shown in Fig. 2. In most cases, native antigen was used for preparing standard curves. However, quantitative analysis with Anti-bSt-16 (specific for a sequence exposed only upon denaturation) could only be performed by preparing a standard curve using denatured/reduced antigen.

Thermal Stability of bSt Epitopes—Initial attempts to measure conformational alterations of bSt using our panel of mAbs focused on the stability of the respective epitopes at various temperatures. Thermal stability profiles of each bSt epitope probed by the mAbs were prepared by performing the CSI at varying temperatures for a fixed period of time (Fig. 3). All six of the epitopes examined demonstrated unique thermal stabilities, suggesting that each antibody recognizes a unique determinant. The unique nature of each epitope was subsequently confirmed by competitive sandwich immunoassays and assays involving bSt analogs (data not shown).

The epitope corresponding to Anti-bSt-1 demonstrated the lowest thermal stability with significant epitope modifications detected at temperatures as low as 45 °C and almost total loss of reactivity at or above 50 °C. In contrast, Anti-bSt-16 was capable of detecting significant changes in bSt structure only at temperatures above 70 °C. The epitope for Anti-bSt-7 remained intact across the entire range of temperatures examined. However, a temperature-dependent loss of reactivity was observed for this epitope when a similar thermal stability experiment was carried out under reducing conditions (data not shown).

These results are consistent with previous studies which showed that Anti-bSt-7 loses reactivity with bSt only after the antigen is denatured under reducing conditions (16). The thermal stability profile generated for the Anti-bSt-4 epitope is initially consistent with that of a partially buried sequence epitope (enhanced reactivity at elevated temperatures), but reactivity was found to decrease at temperatures above 75 °C. Evidence from kinetic studies (below) indicates that the decrease in Anti-bSt-4 epitope reactivity noted after the initial enhancement is not due to epitope denaturation or degradation, but is instead the result of an as yet unexplained assay artifact.

Denaturation Kinetics of bSt Epitopes—Comparing thermal stability profiles of bSt prepared using different denaturation times indicates that the temperature at which conformational alterations occur for each epitope is inversely proportional to the denaturation time used (data not shown). This observation indicates that heat-induced unfolding of bSt is kinetically controlled and does not reflect a temperature-dependent equilibrium. Therefore, denaturation kinetics of the epitopes probed by the mAb panel were investigated. Denaturation kinetics profiles were prepared under reducing and nonreducing conditions for each epitope (Fig. 4). The temperatures used for this analysis were tailored to each epitope based on the thermal stabilities described above. Since different temperatures were used to prepare the denaturation kinetics profiles for different epitopes, the unfolding kinetics for the various epitopes cannot be compared directly. However, the effect of different denaturation conditions (such as reducing versus nonreducing conditions) can be compared for each individual epitope. The rate of unfolding for the epitope corresponding to Anti-bSt-1 was accelerated in the presence of reducing agent. Similar accelerated denaturation kinetics were noted under reducing conditions for Anti-bSt mAbs 2, 3, and 7. The unfolding of bSt to reveal the buried epitope of Anti-bSt-16 was likewise accelerated under reducing conditions.

The denaturation kinetics profiles generated for the Anti-bSt-4 epitope were more complicated than those generated for the other epitopes examined. Under nonreducing conditions, this epitope exhibited a rapid increase in reactivity, presumably due to improved surface accessibility of the partially buried epitope, followed by a slower loss of reactivity to levels below that noted on native bSt. These data agree (qualitatively) with the thermal stability profile described for the Anti-bSt-4 epitope under nonreducing conditions above (Fig. 3). Under reducing conditions, the initial enhancement of reactivity observed for this mAb is greater in magnitude, but the subsequent loss of reactivity does not fall below the reactivity noted with native bSt. Failure of this epitope to reveal a significant loss of reactivity under reducing conditions indicates that the loss noted under the less harsh nonreducing conditions is not a result of denatured or degraded epitope structure. Instead, these results may be indicative of different unfolding pathways under different denaturation conditions resulting in potential differences in antigen/membrane association or covering of the epitope by other regions of the molecule as it unfolds.

Refolding of bSt Epitopes following Thermal Denaturation—The ability of bSt to refold following thermal denaturation in the presence or absence of reducing agent was examined by repeating the denaturation kinetics studies using the reversible CSI mode (Fig. 5). Anti-bSt mAbs 1 and 2 failed to detect any significant refolding of their respective epitopes following
thermal denaturation under reducing conditions. Anti-bSt mAbs 3, 4, 7, and 16 indicate that a significant amount of refolding occurs in some areas of the molecule after reducing denaturation, although refolding is less than complete after "long" denaturation times as indicated by Anti-bSt mAbs 3 and 7. The refolding potential of bSt after nonreducing thermal denaturation was good in the areas of the Anti-bSt-4 and Anti-bSt-16 epitopes. However, the reactivity of the Anti-bSt-1 and Anti-bSt-2 epitopes was actually lower on "refolded" bSt than on bSt trapped in the denatured form using the irreversible CSI mode under the same time and temperature conditions. Similar results were obtained for the Anti-bSt-3 epitope after short denaturation times. These decreased reactivities noted with refolded bSt are statistically significant and suggest that some conformational features of the refolded protein are formed incorrectly.

Verification of CSI Results with NMR Spectroscopy—The results from CSI analysis of bSt suggest that significant conformational modifications are imparted on the bSt molecule in the temperature range of 45–50 °C. In order to confirm that the mAbs were detecting "real" conformational alterations at these low temperatures, we probed the structure of bSt in solution using NMR spectroscopy. Even at these relatively low temperatures, analysis of the NMR spectra is hampered by the solution stability of bSt. A 2 mg/ml solution of bSt is clear at room temperature, slightly cloudy at 37 °C, and heavily precipitated at 55 °C. This behavior alone suggests that significant conformational changes, leading to aggregation and precipitation of bSt, are occurring across this temperature range.

The ^1H spectrum of bSt at 27 °C contains broad regions of overlapping resonances consistent with a folded protein in the 20-kDa molecular mass range. The aromatic region of the ^1H spectrum, at several temperatures, is shown in Fig. 6. Spectra obtained at increased temperatures, (Fig. 6, B → D), illustrate that the protein still contains secondary/tertiary structure at elevated temperatures, but that the structure has changed somewhat from the native structure observed at room temperature. This is illustrated by the resonances labeled I and II (Fig. 6D), which have been assigned previously to the six tyrosine 2',6' and 3',5' aromatic protons, respectively (17). At 50 °C, the average chemical shifts for these resonances are 7.70 and 6.95 ppm, which is consistent with those observed for random coil tyrosine residues (18). While the tyrosine resonances may experience a microenvironment more similar to a denatured protein, the chemical shifts of at least two other residues are not affected by this temperature range. The resonances labeled H and P have been assigned previously to the histidine C2H and phenylalanine aromatic protons, respectively (17, 19). Each of these resonances experience a temperature-induced line narrowing, but do not experience a deviation in chemical shift. This suggests that the microenvironment...
surrounding these residues has not been altered significantly by temperature. Together, these results support the CSI data, suggesting that bSt exists in a partially unfolded state after incubation at 45–50°C for 4–5 min.

**Analysis of Disulfide Bonds—** Experiments designed to examine the kinetics of bSt disulfide bond reduction (under irreversible CSI conditions) and the potential for reoxidation of the disulfide bond in bSt following reducing denaturation (reversible CSI), were performed to further validate the results described above for CSI analysis of bSt. Under irreversible CSI conditions, reduction of the two disulfide bridges in bSt was rapid and complete at 45°C (Fig. 7), and the rate of reduction was accelerated at higher temperatures (data not shown). No apparent reoxidation was observed following reduction under irreversible CSI conditions since the denatured and reduced protein was restrained by covalent interactions with IAV. The loss of disulfide bond structure under irreversible conditions appears to proceed faster than denaturation of the Anti-bSt-7 epitope which is directly stabilized by the disulfide bond formed between cysteine residues at positions 181 and 189 (16). Analysis of disulfide bonds following reversible denaturation CSI treatments revealed a multiphasic reoxidation profile indicating that some disulfide reoxidation is possible when the molecule is not constrained by covalent interactions with IAV. After short reducing denaturation incubations at 45°C, approximately 50% of the disulfide structures had reformed (Fig. 7). The potential for disulfide bond reoxidation was found to diminish as the denaturation incubation was extended. This diminished reoxidation potential appears to parallel a similar decrease in Anti-bSt-7 epitope refolding potential.

**DISCUSSION**

The Conformation-Sensitive Immunoassay (CSI) has been applied to investigate bovine somatotropin (bSt) structure using a panel of bSt-specific antibodies. The CSI is ideally suited for this application because it allows one to: 1) measure quantitative thermal denaturation kinetics of individual epitopes; 2) limit refolding of the test protein after thermal denaturation by covalently trapping regions of the protein as they unfold (irreversible mode); and, 3) characterize conformational features with respect to refolding potential after thermal denaturation (reversible mode). The CSI also eliminates potential aggregation and precipitation commonly observed in solution-based thermal denaturation studies by immobilizing the test protein on a hydrophilic membrane support that minimizes surface denaturation commonly observed with other solid phase immunoassays.

CSI-based thermal stability analysis of bSt revealed a wide range of stabilities for different epitopes. These results suggest that different antibodies are best suited for investigating specific stages of bSt denaturation. The Anti-bSt-1 epitope is the most thermally labile of all the epitopes studied here. This epitope exhibited significant structural modification after incubating bSt at temperatures as low as 45°C for periods as
The degree to which bSt disulfide bonds were reduced following reducing denaturation was determined by probing bSt on IAV for free SH groups using lucifer yellow iodoacetamide. Free SH analysis was performed after reducing denaturation at 45°C under irreversible (●) and reversible (▲) CSI conditions. Results were normalized to a standard bSt titration curve prepared using conditions resulting in complete reduction of disulfide bonds (irreversible CSI denaturation/reduction at 100°C for 5 min).

Previous analysis of the Anti-bSt-7 epitope suggested that this epitope is directly stabilized by the disulfide bond between the cysteine residues at positions 181 and 189 (16). The effects of this stabilization are clearly observed when denaturation kinetics of this epitope under reducing and nonreducing conditions are compared. None of the other conformational epitopes examined with the panel of mAbs were found to be stabilized directly by disulfide bonds since each conformation-specific mAb detected epitope denaturation under both reducing and nonreducing conditions. However, the fact that all of the conformation-specific mAbs, as well as the denatured-specific mAb (Anti-bSt-16), revealed accelerated denaturation kinetics under reducing conditions suggests that the two disulfide bridges of bSt impart some degree of indirect global stabilization to the entire hormone molecule.

A simple exponential decay of bSt disulfide bond structure was observed using lucifer yellow iodoacetamide to probe bSt denaturation under irreversible reducing conditions. However, a biphasic profile would be expected if reduction of the two bonds occurred sequentially. These data suggest, therefore, that reduction of the two bonds occurs concurrently under these conditions. In contrast, reversible CSI analysis of disulfide bond structure did result in a biphasic profile, with approximately 50% of the bonds reforming after relatively short reducing denaturation incubations at 45°C. This observation suggests that either: 1) both disulfide bridges of bSt reoxidize to some degree and that the combined total reoxidation equals 50% of the disulfide bonds in the IAV-bound protein, or 2) one of the two bonds reforms in all of the IAV-bound molecules while the other bridge remains reduced in essentially all molecules. We favor the latter explanation because the Anti-bSt-7 epitope exhibits complete refolding under identical conditions, suggesting that the 181/189 disulfide bridge reoxidizes in essentially all bSt molecules while the 53/164 bond remains reduced. Further support of this explanation is provided by the fact that the loss of reoxidation potential after longer denaturation times correlates with a similar loss of refolding potential for the Anti-bSt-7 epitope (181/189 disulfide bridge).

Analysis of bSt denaturation using Anti-bSt-16 and the reversible CSI mode indicates that significant refolding of bSt is possible after thermal denaturation under reducing and nonreducing conditions. However, several of the epitopes examined in this study failed to exhibit complete refolding under the same conditions. Failure to regain native structure following thermal denaturation was most evident for the epitopes recognized by Anti-bSt mAbs 1 and 2. These epitopes showed little difference between irreversible and reversible denaturation profiles after denaturing under reducing conditions. While these results appear to indicate that no refolding occurred in the areas of these epitopes, it is also likely that some degree of refolding did occur, but that the refolding was not sufficient to restore the specific spatial orientation of antigen domains necessary for antibody binding. Similarly, the elimination of Anti-bSt-16 epitope reactivity following reversible CSI treatment does not imply that the antigen has regained native structure.

![Graph](http://www.jbc.org/)

**Fig. 7. Analysis of disulfide bonds after reducing CSI treatments.** The degree to which bSt disulfide bonds were reduced following reducing denaturation was determined by probing bSt on IAV for free SH groups using lucifer yellow iodoacetamide. Free SH analysis was performed after reducing denaturation at 45°C under irreversible (●) and reversible (▲) CSI conditions. Results were normalized to a standard bSt titration curve prepared using conditions resulting in complete reduction of disulfide bonds (irreversible CSI denaturation/reduction at 100°C for 5 min).
Rather, these results only indicate that the refolding that has occurred was sufficient to cover the Anti-bSt-16 epitope making it inaccessible for antibody binding.

The reasons that bSt fails to refold completely following thermal denaturation are not known. Previous studies have demonstrated that disulfide bond “scrambling” may play a role in irreversible thermal denaturation of some proteins (22). It is unlikely that this is a significant factor in the irreversibility of bSt thermal denaturation since the Anti-bSt-7 epitope, which is dependent on the 181/189 disulfide bridge, demonstrates good reactivity after reversible CSI analysis. Chemical modifications induced at elevated temperatures have also been identified as possible causes for irreversible denaturation of proteins (21). These modifications generally occur at temperatures higher than those used for denaturation of conformational features in the CSI and also generally require longer incubations than those employed here. Therefore, it is unlikely that chemical alterations of bSt are responsible for the refolding problems. Likewise, the aggregation of proteins that commonly occurs during thermal denaturation studies also cannot explain the irreversible thermal denaturation of bSt since the test protein was immobilized on a solid support during denaturation. A more plausible explanation of the irreversible thermal denaturation of proteins such as bSt has been offered by Klibanov and Mozhaev (23) after investigating thermal denaturation of immobilized trypsin. Their data support the theory that non-native intramolecular interactions occur during refolding of thermally denatured proteins and that these interactions remain after the protein is cooled to ambient temperatures. Once cooled, the presence of these non-native interactions may prevent the protein from forming its native structure spontaneously. The presence of such non-native interactions may explain the apparently incorrect refolding of bSt detected in the areas of the Anti-bSt-1 and Anti-bSt-2 epitopes when the reversible CSI was performed under nonreducing conditions.

The data presented in this report enhance our understanding of bSt structure by probing the denaturation and renaturation behavior of specific conformational features of the molecule. We have previously reported the ability of the CSI to correctly characterize conformation-sensitive monoclonal antibodies to proteins other than bSt (16). Taken together, these reports suggest that the methods described here may be generally applicable to the study of denaturation and renaturation behavior of globular proteins. In particular, however, we have demonstrated the utility of these techniques for the analysis of proteins, like bSt, that demonstrate poor solubility upon denaturation.

Acknowledgments—We thank Greg Cavey for technical assistance related to disulfide bond analysis and Dr. Gary Martin for the editorial expertise that he provided during preparation of this manuscript.

REFERENCES

1. Murry-Brelier, A., and Goldberg, M. E. (1988) Biochemistry 27, 7633–7640
2. Allen, M. J., Emmerson, R., and Nall, B. T. (1994) Biochemistry 33, 3967–3973
3. Collawn, J. F., Wallace, C. J. A., Proudfoot, A. E. I., and Patterson, Y. (1988) J. Biol. Chem. 263, 8625–8634
4. Fedorov, A. N., Friguet, F., Djavadi-Ojhani, L., Alakhov, Y. B., and Goldberg, M. E. (1992) J. Mol. Biol. 228, 351–358
5. Santome, J. A., Delia, J. M., Paladini, A. C., Peña, C., Bisciglio, M. J., Daurat, S. T., Poskus, E., and Wolfenstein, C. E. M. (1973) Eur. J. Biochem. 37, 164–170
6. Wallis, M. (1973) FEBS Lett. 35, 11–14
7. Bell, J. A., Moffat, K., Vanderhaer, B. K., and Golde, D. W. (1985) J. Biol. Chem. 260, 8520–8525
8. Brems, D. N., Plaisted, S. M., Havel, H. A., Kauffman, E. W., Stodola, J. D., Eaton, L. C., and White, R. D. (1985) Biochemistry 24, 7662–7668
9. Havel, H. A., Kauffman, E. W., Plaisted, S. M., and Brems, D. N. (1986) Biochemistry 25, 6335–6338
10. Holzman, T. F., Brems, D. N., and Dougherty, J. J. (1986) Biochemistry 25, 6907–6917
11. Brems, D. N., Plaisted, S. M., Kauffman, E. W., and Havel, H. A. (1986) Biochemistry 25, 6339–6343
12. Brems, D. N., Plaisted, S. M., Dougherty, J. J., and Holzman, T. F. (1987) J. Biol. Chem. 262, 2590–2596
13. Chen, C. H., and Sonnberg, M. (1977) Biochemistry 16, 2110–2118
14. Brems, D. N., Plaisted, S. M., Kauffman, E. W., Lund, M. E., and Lehrman, S. R. (1987) Biochemistry 26, 7774–7778
15. Davio, S. R., and Hageman, M. J. (1993) in Stability and Characterization of Protein Peptide Drugs: Case Histories (Wang, Y. J., and Pearlman, R., eds) pp. 59–89, Plenum Publishing Corp., New York
16. Pfund, W. P., and Bourdage, J. S. (1990) Mol. Immunol. 27, 495–502
17. Holzman, T. F., Dougherty, J. J., Brems, D. N., and MacKenzie, N. E. (1990) Biochemistry 29, 1255–1261
18. Wuthrich, K. (1986) NMR of Proteins and Nucleic Acids, p. 71, Wiley-Interscience, New York
19. Mackenzie, N. E., Plaisted, S. M., and Brems, D. N. (1989) Biochim. Biophys. Acta 99, 166–171
20. Secchi, C., Berrini, A., Borromeo, V., Gamba, D., and Broggi, E. (1991) Hybridoma 10, 489–505
21. Volkin, D. B., and Middaugh, C. R. (1992) in Stability of Protein Pharmaceuticals (Ahern, T. J., and Manning, M. C., eds) Part A, pp. 215–247, Plenum Publishing Corp., New York.
22. Zale, S. E., and Klibanov, A. M. (1986) Biochemistry 25, 5432–5444
23. Klibanov, A. M., and Mozhaev, V. V. (1978) Biochem. Biophys. Res. Commun. 83, 1012–1014
Structural Analysis of Bovine Somatotropin Using Monoclonal Antibodies and the Conformation-Sensitive Immunoassay
William P. Pfund, James S. Bourdage and Kathleen A. Farley

J. Biol. Chem. 1996, 271:14055-14061.
doi: 10.1074/jbc.271.24.14055

Access the most updated version of this article at http://www.jbc.org/content/271/24/14055

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 20 references, 3 of which can be accessed free at http://www.jbc.org/content/271/24/14055.full.html#ref-list-1