Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 34 (2006) 131–141
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Total chemical synthesis of the B1 domain of protein L from *Peptostreptococcus magnus*

Xiaoye Yang, Michael C. Fitzgerald *

Department of Chemistry, Duke University, Durham, NC 27708, USA

Received 11 February 2006

Abstract

Reported here is a native chemical ligation strategy for the total chemical synthesis of the B1 domain of protein L. A synthetic construct of this 76 amino acid protein domain was prepared by the chemoselective ligation of two unprotected polypeptide fragments, one containing an N-terminal cysteine residue and one containing a C-terminal thioester moiety. The polypeptide fragments utilized in the ligation reaction were readily prepared by stepwise solid phase peptide synthesis (SPPS) methods for Boc-chemistry. The milligram quantities of protein required for conventional biophysical studies were readily accessible using the synthetic protocol described here. The folding properties of the synthetic protein L construct were also determined and found to be very similar to those of a similar wild-type protein L constructs prepared by recombinant-DNA methods. This work facilitates future unnatural amino acid mutagenesis experiments on this model protein system to further dissect the molecular basis of its folding and stability.

Keywords: Protein L; Native chemical ligation; Protein folding; Solid phase peptide synthesis

1. Introduction

Protein L is a multidomain protein expressed on the surface of some strains of the anaerobic bacterial species *Peptostreptococcus magnus* [1]. The B1 domain of protein L

* Corresponding author. Fax: +1 919 660 1605.
E-mail address: michael.c.fitzgerald@duke.edu (M.C. Fitzgerald).

0045-2068/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bioorg.2006.02.001

is a small 76-residue immunoglobulin G (IgG) binding domain (see Fig. 1). The folded, three-dimensional structure of the B1 domain of protein L has been determined by NMR methods and X-ray crystallographic methods [2–5]. The structural data revealed that the first 15 residues of the domain are largely disordered and that the remaining residues in the protein fold into a ββαββ structure in which the α-helix is packed against a four-stranded β-sheet.

The B1 domain of protein L has served as a useful model system to study protein folding and stability; and as such, it has been the subject of extensive biophysical studies to determine the thermodynamic and kinetic properties of its folding reaction [6–10]. In these biophysical studies, the protein folding/unfolding reaction was found to be well-modeled by a two-state process; and the thermodynamic and kinetic effects of some 70 different point mutations on the folding properties of this protein domain were assessed. Interestingly, the results of these site-directed mutagenesis experiments revealed a well-defined transition state in the protein's folding pathway. In this transition state, the amino acid side chains in the first β-hairpin turn in the protein's three-dimensional structure were found to be in native-like confirmations [8–10].

The biophysical studies on the B1 domain of protein L performed to date have all focused on understanding the role of individual amino acid side-chain functionalities in the protein's folding and stability. The role of the polypeptide backbone (e.g., backbone–backbone hydrogen bonding interactions) has not been directly studied in this system. This is mainly because such studies require modification of the protein's polypeptide backbone, which is not easily accomplished using conventional, biologically based, and site-directed-mutagenesis methods. In the past decade, total chemical synthesis strategies have become an attractive means by which to introduce mutations into the polypeptide backbone of proteins. For example, the replacement of an amide bond with an ester bond has proven to be a backbone mutation that is easily accessible by chemical synthesis methods, and this mutation has been introduced into several different protein systems in order to study the role of backbone hydrogen bonding interactions in protein folding and function [11].

An important first step in unnatural amino acid mutagenesis experiments using total chemical synthesis strategies is the establishment of a synthetic protocol for the wild-type protein. Reported here is such a protocol for the total chemical synthesis of the B1 domain of protein L. The protocol employs the native chemical ligation strategy pioneered by

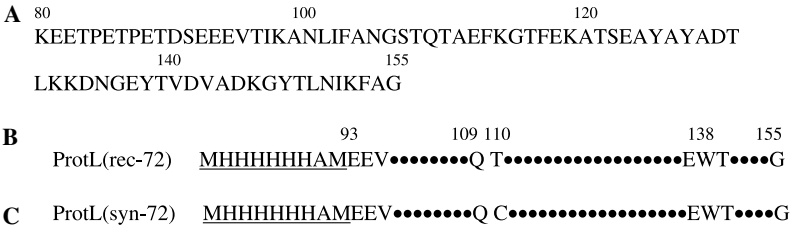

Fig. 1. Amino acid sequences of the protein L constructs referred to in this work. (A) Complete primary amino acid sequence of the B1 domain of protein L. (B) and (C) show schematic representations of the primary amino acid sequences of the recombinant and synthetic constructs, respectively, that were studied in this work. The numbering follows that in [2]. The underlined sequences are not part of the wild-type sequence; they were added in earlier work when the protein was cloned and overexpressed in *E. coli*.

Kent and co-workers [12,13], and accordingly, the full length B1 domain of protein L is prepared here by the chemoselective ligation of two unprotected polypeptide fragments. The unprotected polypeptide fragments utilized in the ligation reaction were readily prepared by highly optimized, stepwise solid phase peptide synthesis (SPPS) methods for *tert*-butoxycarbonyl(Boc)-chemistry. The milligram quantities of protein required for conventional biophysical studies are readily accessible using the synthetic protocol described here. The results of our preliminary biophysical studies on the synthetic protein L constructs prepared here also indicate that the folding properties of the synthetic material are very similar to those of similar wild-type protein L constructs prepared by recombinant-DNA methods.

2. Materials and methods

2.1. Materials

Boc-L-amino acids and *S*-trityl-β-mercaptopropionic were purchased from Peptide Institute Inc. and NovaBiochem. All Boc-amino acid-4-(oxymethylphenylacetamidomethyl) resins and *p*-methylbenzoylhydroxylamine (MBHA) resin were obtained from Applied Biosystems. The 2-(*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uroniumhexafluorophosphate (HBTU) was purchased from Quantum Biotechnologies. Trifluoroacetic acid (TFA) was from Halocarbon and HF was obtained from Matheson Gas Products, Inc. DMF and DCM were purchased from Fisher Scientific. All solvents were of high purity, suitable for peptide synthesis and HPLC and used without further purification.

The recombinantly generated ProtL construct in this work, ProtL(rec-72), was obtained after its overexpression in *Escherichia coli* cells and subsequent purification as previously described [14]. The protein L clone used to generate the ProtL(rec-72) material in this work was kindly provided by Professor David Baker.

2.2. Instrumentation

Analytical and semi-preparative reversed-phase HPLC were performed on a Dyvamax-Rainin dual-pump high-pressure mixing system with UV–visible absorbance detector. Semi-preparative HPLC was run on a Vydac C18 or C4 column (10 μm, 10×250 mm) at a flow rate of 3 ml/min. Analytical HPLC was performed on a Vydac protein/peptide C18 or C4 column (5 μm, 4.6×150 mm) at a flow rate of 1 ml/min. All runs used linear gradients of 0.1% TFA in water (buffer A) vs. 90% acetonitrile/10% water plus 0.1% TFA (buffer B).

Electrospray ionization mass spectrometry was performed on a PE Sciex API 150EX. Peptide masses were calculated from the experimentally observed m/z values of the observed protonation states of each peptide sample using the MacProMass™ program.

All circular dichroism (CD) measurements were made at 295 K using an Applied Photophysics PiStar 180 CDF spectrometer fitted with a temperature controlled cell holder. CD spectra were recorded in a 50 mM sodium phosphate buffer (pH 7.0) using a 300 μl cuvette.

Protein concentrations were determined based on their UV absorbance at 280 nm and a molar extinction coefficient of 9970 M^{-1} cm^{-1}[7]. UV spectra were recorded on a Hewlett Packard 8452 A diode array UV–visible spectrophotometer.

2.3. Peptide fragment synthesis

All peptides were manually prepared using highly optimized solid phase peptide synthesis protocols for Boc-chemistry as described previously [15]. The C-terminal thioester-containing peptides were prepared using a $HSCH_2CH_2CO$-Leu-OCH_2-MBHA resin as previously described [16]. Peptide fragments bearing a carboxyl group at their C-terminus were prepared on the appropriate PAM resin.

The side-chain protecting groups employed in this work were as follows: Arg(*p*-tolylsulfonyl). Asn(xanthyl), Cys(4-methylbenzyl), Glu(*O*-cyclohexyl), His(Bom), Leu(H_2O), Lys(2-Cl-Z), Ser(benzyl), Thr(benzyl), Trp(Hoc), Tyr(Br-Z). After chain assembly was complete, peptides were deprotected and cleaved from the resin by treatment with anhydrous HF in the presence of *p*-cresol (5%, v/v) at 0 °C for 1 h. In the case of His-containing peptides, 30% (w/w) resorcinol was also present during the HF treatment. Following evaporation of the HF under reduced pressure, the crude synthetic peptides were precipitated, washed with chilled anhydrous diethyl ether, and dissolved in a water/acetonitrile solution (30/70, v/v) containing 0.1% TFA. The resulting solution was diluted with water and lyophilized. Ultimately, the lyophilized synthetic products were purified by semi-preparative RP-HPLC using either a C18 or a C4 column, depending on the size of the polypeptide fragment. The pure-peptide-containing fractions (as determined by ESI mass spectrometry) in each semi-preparative RP-HPLC purification were combined, diluted with water, and lyophilized.

2.4. Native chemical ligation reactions

The pure, lyophilized, peptide fragments from above were used in the native chemical ligation reactions in this work. Ligation reactions were carried out in 0.1–1.0 ml volumes of a reaction buffer containing 6 M GdmCl and 100 mM sodium phosphate (pH 7.5). Typically, the N-terminal Cys-containing fragment and the C-terminal thioester-containing fragment were dissolved in the reaction buffer at concentrations of 1 and 1.5 mM, respectively, and thiophenol, 4% (v/v), was added to initiate the chemical ligation reaction. The ligation reactions were essentially complete (as judged by analytical RP-HPLC) in 18 h. The full-length protein L constructs were purified from the reaction mixtures using semi-preparative RP-HPLC in which the C4 column and a linear gradient of 20–60% buffer B in Buffer A over 60 min were employed for the separation.

2.5. Thermodynamic analysis of protein folding/unfolding

Far UV-CD was used to monitor the GdmCl-induced equilibrium unfolding properties of the protein L constructs studied here. These experiments involved the preparation of stock solutions of folded and unfolded protein samples. The protein concentration (10 μM) and buffer composition (50 mM sodium phosphate, pH 7) of the folded and unfolded stock solutions were identical except that the unfolded stock solution contained 8 M GdmCl. Denaturation curves were generated by adding the unfolded stock solution to the folded stock solution using the automatic titrator on the PiStar 180 CDF spectrometer. At each point in the titration, the sample in the 2 ml cuvette was allowed to equilibrate for 3 min before the CD signal was monitored at 222 nm.

The equilibrium unfolding reaction for the B1 domain of protein L has previously been shown to be well-modeled by a two-state process involving only folded (N) and unfolded (U) species [6,7].

$$\mathrm{N} \leftrightarrow \mathrm{U} \tag{1}$$

Therefore, the CD denaturation curves in this work were analyzed according to a two-state model in order to extract a folding free energy, ΔG_{U} and m-values. In this analysis, the raw CD signal in the unfolding experiments on both synthetic and recombinant constructs were normalized with respect to the fraction of unfolded protein (f_{U}), where

$$f_{\mathrm{U}} = ([\theta]_{\mathrm{U}} - [\theta])/([\theta]_{\mathrm{N}} - [\theta]_{\mathrm{U}}) \tag{2}$$

In Eq. (2), $[\theta]$ is the observed ellipticity at 222 nm, $[\theta]_{\mathrm{U}}$ is the ellipticity of the unfolded state, and $[\theta]_{\mathrm{N}}$ is the ellipticity of the folded state. The equilibrium constant for the unfolding reaction can be expressed as a ratio between the fraction of folded monomer, f_{N}, and the fraction of unfolded monomer, f_{U}

$$K_{\mathrm{U}} = f_{\mathrm{U}}/f_{\mathrm{N}} \tag{3}$$

This allows the free energy of unfolding of the protein at each denaturant concentration, ΔG_{app}, to be calculated according to Eq. (4)

$$\Delta G_{\mathrm{app}} = -RT \ln(f_{\mathrm{U}}/f_{\mathrm{N}}), \tag{4}$$

where R is the gas rate constant and T is the temperature. Values for ΔG_{app} were calculated at each point in the transition region of the unfolding curve (i.e., at f_{U} values between 0.9 and 0.1). The ΔG_{app} values were plotted against the [GdmCl], and the data were fit to Eq. (5) using a linear-least squares analysis.

$$\Delta G_{\mathrm{app}} = \Delta G_{\mathrm{U}} - m[\mathrm{GdmCl}] \tag{5}$$

Ultimately, ΔG_{U} and m-values were taken from the y-intercepts and slopes determined in our linear-least squares analyses [17].

2.6. Kinetic analysis of protein folding/unfolding

The kinetic folding and unfolding experiments were performed on the Applied Photophysics Pi-Star 180 CDF spectrometer system in the stopped-flow fluorescence mode. The temperature was maintained at 295 K using a circulating water bath. An excitation wavelength of 280 nm and a 305 nm cut-off emission filter were used to make the fluorescence measurements. Refolding reactions were performed by 1:10 dilution of denatured protein (~50 μM) into refolding buffer solution containing 50 mM sodium phosphate, pH 7.0. The final GdmCl concentration ranged between 0.3 and 2.7 M. Unfolding was initiated by 1:10 dilution of folded protein into unfolding buffer containing 50 mM sodium phosphate, pH 7.0, and a high concentration of GdmCl. The final concentration of GdmCl in these unfolding experiments ranged from 2.0 to 6.0 M. The kinetic traces (i.e., fluorescence signal vs. time plots) at each GdmCl concentration were analyzed using a single exponential fitting procedure to obtained a single exponential rate constant, k_{obs}. In total, the k_{obs} values from five different kinetic traces were averaged, and these average value were fit to a two-state model using the following equation as described previously [7]:

$$\ln k_{\mathrm{obs}} = \ln[k_{\mathrm{F}}^{\mathrm{H_2O}} \exp(-m_{\mathrm{F}}[\mathrm{GdmCl}]) + k_{\mathrm{U}}^{\mathrm{H_2O}} \exp(m_{\mathrm{U}}[\mathrm{GdmCl}])], \tag{6}$$

where $k_F^{H_2O}$ and $k_U^{H_2O}$ are the rates of folding and unfolding in the absence of denaturant, and m_U and m_F are the slopes of the unfolding and refolding reactions, respectively.

3. Results and discussion

3.1. Design

The goal of this work was to obtain synthetic access to the B1 domain construct that has been the subject of extensive biophysical studies by Baker and co-workers [5–10]. This construct, which is shown schematically in Fig. 1B, encompasses residues 93–155 of the B1 domain amino acid sequence shown in Fig. 1A, and it contains a nine amino acid His-tag sequence at the protein's N-terminus as well as a Tyr to Trp mutation at position 138. The Tyr to Trp mutation was previously found to facilitate biophysical studies of the protein's folding reaction using fluorescence spectroscopy as a structural probe [7]. Residues 80–92 in the B1 domain sequence shown in Fig. 1A were deleted in the Baker group's construct as they were found to be disordered in the NMR structure [2]. The His-tag incorporated into the Baker group's construct greatly facilitated purification of the protein that was obtained by recombinant-DNA methods. The His-tag sequence was also found to have little or no effect on the folding properties of the rest of the protein domain [7]. While it was not the initial goal of this study to include the His-tag sequence in our synthetic construct, the incorporation of this sequence into our synthetic construct turned out to be a useful design feature (see below).

Proteins and protein domains in 40–70 amino acid size range have been synthesized by stepwise solid phase synthesis methods. However, we found the 63 structured amino acids in the B1 domain of protein L (see residues 93–155 in Fig. 1A) were not readily assembled in high yield using the highly optimized SPPS protocols for Boc-chemistry described above in Section 2. The overall yield and quality of the final product obtained after SPPS were low, and RP-HPLC purification of the crude synthetic product was unproductive. This result prompted us to explore a native chemical ligation strategy for the total chemical synthesis of the B1 domain of protein L.

The native chemical ligation strategy pursued here required a cysteine residue at the ligation site [12,13]. While there are no Cys residues in the wild-type amino acid sequence of the B1 domain of protein L, several Cys-containing mutants of the B1 domain of protein L were constructed in an earlier biophysical study of the protein [18]. In this earlier study, Cys mutations were incorporated into the protein's primary amino acid sequence at positions 94, 110, and 139 of the amino acid sequence shown in Fig. 1A, and none of the these Cys mutations were found to significantly alter the protein's folding and stability [18]. This prompted the incorporation of a Thr to Cys mutation at position 110 in our synthetic constructs in order to create a Gln-Cys ligation site (see Fig. 1C). The use of this Gln-Cys ligation site in our total chemical synthesis of the B1 domain of protein L required the stepwise SPPS synthesis of two polypeptide fragments including a 26 amino acid residue C-terminal-thioester-containing fragment, ProtL(MH_6AME^{93}-Q^{109})COSR, and a 46 amino acid residue N-terminal-Cys-containing fragment, ProtL(C^{110}-G^{155}).

In theory, the His-tag sequence in the ProtL(rec-72) construct (see the nine underlined residues in Fig. 1B) could have been deleted in our synthetic construct. It was incorporated into the Baker group's recombinant construct to facilitate the protein's purification using a nickel column. The RP-HPLC purification strategy employed here for the synthetic

protein did not require the nickel binding affinity of the His-tag sequence. However, we found that inclusion of the His-tag sequence in our synthetic construct was important for the success of our synthetic strategy as it greatly enhanced the solubility properties of the C-terminal-thioester-containing fragment in our ligation strategy. For example, the stepwise SPPS of a ProtL(M-E^{93}-Q^{109})COSR fragment without the His-tag was attempted, but it proved to be especially problematic due to the fragment's apparently poor solubility in the various solvents (e.g., water, acetonitrile, and TFA) that are commonly used for the isolation and purification of synthetic peptides. This poor solubility severely limited the yield of full-length fragment that could be recovered for subsequent use in the chemical ligation reaction.

3.2. Synthesis

The synthetic protein L construct in Fig. 1C required the synthesis of two polypeptide fragments including the C-terminal ligation fragment, ProtL(C^{110}-G^{155}), and the N-terminal ligation fragment, ProtL(MH_6AME^{93}-Q^{109})COSR. Both fragments were readily synthesized using optimized SPPS protocols for Boc-chemistry that have been described elsewhere [15]. The desired peptide products were also readily purified using semi-preparative RP-HPLC and a C18 or C4 column. The C18 column was used for the RP-HPLC purification of the shorter ProtL(MH_6AME^{93}-Q^{109})COSR fragment and the C4 column was used in the case of the longer ProtL(C^{110}-G^{155}) fragment. The overall yields of RP-HPLC-purified peptide fragments were 4.5% and 20% for the ProtL(C^{110}-G^{155}) and ProtL(MH_6AME^{93}-Q^{109})COSR fragments, respectively.

Native chemical ligation reactions were performed with the RP-HPLC-purified ProtL(MH_6AM-E^{93}-Q^{109})COSR and ProtL(C^{110}-G^{155}) fragments to generate the ProtL(syn-72) product. The time course of the reaction was monitored by analytical RP-HPLC (see Fig. 2). The ligation reaction to generate the ProtL(syn-72) product was judged to be nearly complete after 18 h, as determined by a RP-HPLC analysis of the reaction mixture (see Fig. 2B) which indicated an almost complete disappearance of the limiting reactant, the ProtL(C^{110}-G^{155}) fragment, at 18 h. The ProtL(syn-72) product in the ligation reaction mixture was readily purified from the reaction mixture using RP-HPLC with a semi-preparative C4 column. The isolated yield of the RP-HPLC-purified full length ProtL(syn-72) product was ~20%. A RP-HPLC chromatogram and ESI mass spectra recorded for the purified synthetic products are shown in Figs. 2C and D, respectively.

3.3. Biophysical characterization

The three-dimensional structure, thermodynamic, and kinetic properties of the ProtL(syn-72) construct prepared in this work were compared to those properties of a similar protein L construct, ProtL(rec-72), that was generated by recombinant-DNA methods. Note that the only difference between our synthetic construct, ProtL(syn-72), and the recombinant construct, ProtL(rec-72), is that the synthetic construct contained a Thr to Cys mutation at position 110 (see Fig. 1). Far-UV-CD spectra were obtained for the ProtL(syn-72) and ProtL(rec-72) constructs, and the spectra were found to be very similar to each other (see Fig. 3A). These results suggest, as expected from the results in [18], that the Thr to Cys mutation in our synthetic construct did not significantly perturb the folded three-dimensional structure of the B1 domain of protein L.

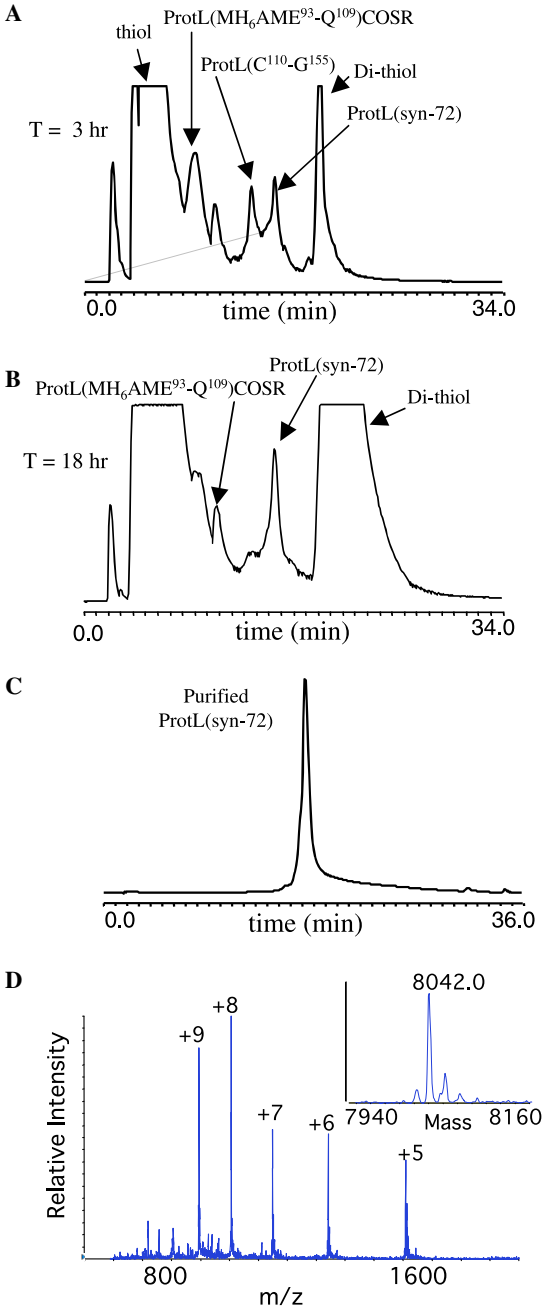

Fig. 2. Analytical characterization of synthetic ProtL construct. Analytical RP-HPLC chromatograms of the ProtL(syn-72) ligation reaction after (A) 3 h and (B) 18 h. (C) Analytical RP-HPLC of purified synthetic ProtL(syn-72). (D) ESI mass spectra recorded for ProtL(syn-72). The inset represents the hypermass reconstruction of the raw MS data. The calculated mass is shown. A 20–60% gradient of acetonitrile in water containing 0.1% TFA was used in each RP-HPLC analysis.

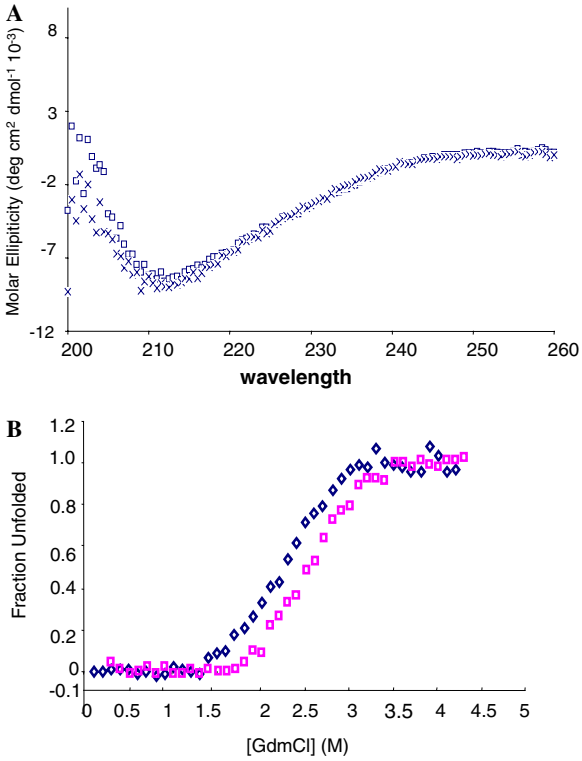

Fig. 3. Biophysical characterization of synthetic ProtL construct. (A) Far UV-CD spectra recorded for the ProtL(rec-72) (opened squares) and ProtL(syn-72) (crosses) constructs. (B) GdmCl-induced equilibrium unfolding curves recorded for the ProtL(rec-72) (squares) and ProtL(syn-72) (diamonds) constructs.

The thermodynamic stabilities of the ProtL(syn-72) and ProtL(rec-72) constructs in this work were also experimentally determined using chemical denaturant-induced equilibrium unfolding experiments, and they were compared to similar determinations previously reported for the recombinantly generated wild-type protein [7]. The CD denaturation curves recorded for the ProtL(syn-72) and ProtL(rec-72) constructs were very similar (see Fig. 3B), with only a small shift ($\sim$0.3 M) detected in the transition midpoint (i.e., $C_{1/2}$) of the ProtL(syn-72) curve ($C_{1/2} = 2.25 \pm 0.04$ M) relative to the ProtL(rec-72) curve ($C_{1/2} = 2.53 \pm 0.02$ M).

The data in Fig. 3B were fit to a two-state folding model in order to evaluate the thermodynamic parameters (i.e., ΔG_F and *m*-values) associated with the protein folding reactions of the synthetic and recombinant constructs in this study. The ΔG_F and *m*-values obtained for each protein construct are summarized in Table 1. The thermodynamic parameters obtained in our CD experiments were very similar for the two constructs studied in this work. The ΔG_F and *m*-values obtained in our CD experiments were also similar to ΔG_F and *m*-values previously reported in [7] for the same ProtL(rec-72) construct under similar conditions by the Baker group (see Table 1).

The unfolding and refolding kinetics of the synthetic and recombinant protein L constructs in this work were also assessed in stopped-flow fluorescence experiments. The

Table 1
Thermodynamic parameters determined for the protein L constructs in this work

	m[a] (kcal mol^{-1} M^{-1})	$\Delta G_{U-F}^{H_2O}$[a] (kcal mol^{-1})
Literature[b]	1.90 ± 0.07	4.6 ± 0.2
ProtL(rec-72)	1.84 ± 0.07	4.7 ± 0.2
ProtL(syn-72)	1.94 ± 0.04	4.4 ± 0.2

[a] Calculated by fitting GdmCl-induced denaturation data to Eq. (5). Reported errors are the standard errors of fitting.
[b] Data on ProtL(rec-72) from Baker and co-workers [7].

kinetic data obtained for folding and unfolding reactions of each construct were obtained at a range of different GdmCl concentrations (see Fig. 4). The $k_F^{H_2O}$, $k_U^{H_2O}$, $m_U^{H_2O}$, and $m_F^{H_2O}$ values extracted from these kinetic experiments are summarized in Table 2. The ΔG_F and m values calculated from the kinetic data are also summarized in Table 2; and they are very similar to those values calculated from the thermodynamic data (see Table 1), providing evidence that the folding/unfolding reactions of each construct in this work are indeed well-described by a two-state folding model.

The biophysical data in Tables 1 and 2 show that the kinetic and thermodynamic parameters obtained for the recombinantly generated wild-type control were essentially identical to those previously reported by Baker and co-workers on the same wild-type construct [7]. A comparison of the kinetic and thermodynamic parameters obtained for the ProtL(rec-72) and ProtL(syn-72) constructs reveals, as expected from the data in [18], that the Thr to Cys mutation in the synthetic construct only had a small destabilizing effect on the protein.

In conclusion, we have developed a native chemical ligation strategy for the total chemical synthesis of the B1 domain of protein L. The biophysical data presented here suggest that the synthetic protein L construct prepared here folded into a native-like structure. Importantly, the Thr to Cys mutation in the synthetic protein L construct in this study did not cause large-scale perturbations to the protein's three-dimensional structure. Nor

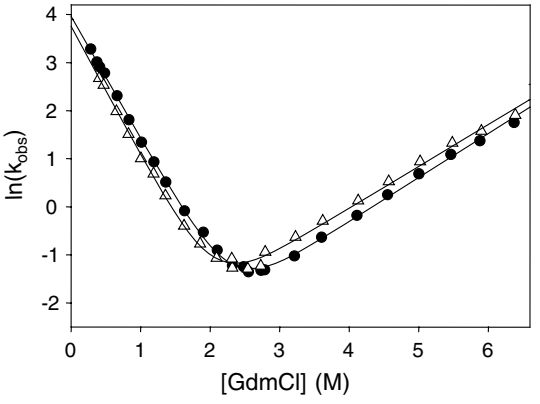

Fig. 4. Kinetic analysis of synthetic ProtL construct. GdmCl dependence of folding and unfolding rates determined in stopped-flow fluorescence experiments on ProtL(rec-72) (opened triangles) and ProtL(syn-72) (closed circles).

Table 2
Kinetic parameters determined for the protein L constructs in this work

	$-m_F$ (kcal mol^{-1} M^{-1})	m_U (kcal mol^{-1} M^{-1})	$k_F^{H_2O}$ (s^{-1})	$k_U^{H_2O}$ (s^{-1})	$m = m_F + m_U$ (kcal mol^{-1} M^{-1})	ΔG_U (kcal mol^{-1})
Literature[a]	1.5 ± 0.01	0.51 ± 0.03	60.6 ± 3.6	0.020 ± 0.002	2.0 ± 0.02	4.7 ± 0.1
ProtL(rec-72)	1.49 ± 0.03	0.54 ± 0.02	52.4 ± 1.8	0.019 ± 0.001	2.03 ± 0.04	4.6 ± 0.1
ProtL(syn-72)	1.57 ± 0.07	0.52 ± 0.03	42.6 ± 3.1	0.027 ± 0.002	2.08 ± 0.08	4.3 ± 0.1

[a] Data on ProtL(rec-72) from Baker and co-workers [7]. All errors are the standard errors of fitting.

did the mutation significantly change the thermodynamic and/or kinetic parameters associated the protein's folding/unfolding reaction. The synthetic protocol developed here is expected to facilitate unnatural amino acid mutagenesis experiments on the B1 domain of protein L to further dissect the folding properties of this widely used model system for protein folding studies.

Acknowledgment

This work was supported by a Grant from the National Institutes of Health (RO1-GM61680) to M.C.F.

References

[1] W. Kastern, E. Holst, E. Nielsen, U. Sjobring, L. Bjorck, Infect. Immun. 58 (1990) 1217–1222.
[2] M. Wikstrom, U. Sjobring, W. Kastern, L. Bjorck, T. Drakenberg, S. Forsen, Biochemistry 32 (1993) 3381–3386.
[3] M. Wikstrom, T. Drakenberg, S. Forsen, U. Sjobring, L. Bjorck, Biochemistry 33 (1994) 14011–14017.
[4] J.W. O'Neill, D.E. Kim, D. Baker, K.Y.J. Zhang, Acta Crystallogr. D57 (2001) 480–487.
[5] K. Johnsen, J.W. O'Neill, D.E. Kim, D. Baker, K.Y.J. Zhang, Acta Crystallogr. D56 (2000) 506–508.
[6] Q. Yi, D. Baker, Protein Sci. 5 (1996) 1060–1066.
[7] M.L. Scalley, Q. Yi, H. Gu, A. McCormack, J.R. Yates, D. Baker, Biochemistry 36 (1997) 3373–3382.
[8] H. Gu, D. Kim, D. Baker, J. Mol. Biol. 274 (1997) 588–596.
[9] D.E. Kim, Q. Yi, S.T. Gladwin, J.M. Goldberg, D. Baker, J. Mol. Biol. 284 (1998) 807–815.
[10] D.E. Kim, C. Fisher, D. Baker, J. Mol. Biol. 298 (2000) 971–984.
[11] X.Y. Yang, M. Wang, M.C. Fitzgerald, Bioorg. Chem. 32 (2004) 438–449.
[12] P.E. Dawson, T.W. Muir, L. Clark-Lewis, S.B.H. Kent, Science 266 (1994) 776–779.
[13] T.W. Muir, P.E. Dawson, S.B.H. Kent, Methods Enzymol. 289 (1997) 266–298.
[14] H. Gu, Q. Yi, S.T. Bray, D.S. Riddle, A.K. Shiau, D. Baker, Protein Sci. 4 (1995) 1108–1117.
[15] M. Schnölzer, P. Alewood, A. Jones, D. Alewood, S.B.H. Kent, Int. J. Pept. Protein Res. 40 (1992) 180–193.
[16] T.M. Hackeng, J.H. Griffin, P.E. Dawson, Proc. Natl. Acad. Sci. USA 96 (1999) 10068–10073.
[17] C.N. Pace, Methods Enzymol. 131 (1986) 266–280.
[18] M.L. Scalley, S. Nauli, S.T. Gladwin, D. Baker, Biochemistry 38 (1999) 15927–15935.