Amide to Ester Substitution in Coiled Coils – The Effect of H-Bond Elimination on Protein Structure Formation

Jessica A. Scheike, Carsten Baldauf, Jan Spengler, Fernando Albericio, M. Teresa Pisabarro, Beate Koksch*

A. - Peptide synthesis and characterization

The peptide and depsipeptides were synthesized using Boc-SPPS.1, 2 The optimized protocols for the machine assisted synthesis (433A Peptide Synthesizer, Applied Biosystems) will be reported elsewhere [J. Spengler, B. Koksch, F. Albericio, manuscript in preparation]. The compounds were purified by semipreparative HPLC and characterized by analytical HPLC and MALDI. Analytic HPLC uses a HPLC Waters 1525, an automatic injector 717 plus and a detector UV-Vis Waters 2487. The column Nucleosil C\textsubscript{18} (250 x 4 mm) was run with acetonitrile (0.036\% TFA) and water milipore (0.045\% TFA). Data were managed with Breeze v3.20 software. In semipreparative scale, the HPLC used was the model Waters 600, the automatic injector Waters 2700, the detector UV-Vis Waters 2487. Samples were collected with the Waters Fraction Collector II. The column Symmetry C\textsubscript{18} (100 x 30 mm) was run with acetonitrile (0.05\% TFA) and water millipore (0.1\% TFA). Data were managed with MassLynux 3.5 software. For MALDI-analysis, the samples were prepared with the ACH-matrix (alpha-cyano-4-hydroxy cinnamic acid, Aldrich). The spectra were obtained with a 4700 Proteomics Analyzer (Applied Biosystems).

<table>
<thead>
<tr>
<th>peptide</th>
<th>MALDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>pp</td>
<td>2892.26 M+H(^+) (exact mass: 2891.73)</td>
</tr>
<tr>
<td>L5(\lambda)</td>
<td>2893.93 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>A10(\alpha)</td>
<td>2893.58 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>K11(\kappa)</td>
<td>2893.49 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>L12(\lambda)</td>
<td>2893.87 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>A13(\alpha)</td>
<td>2894.01 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>L22(\lambda)</td>
<td>2893.39 M+H(^+) (exact mass: 2892.72)</td>
</tr>
<tr>
<td>L12(\lambda), A13(\alpha)</td>
<td>2894.93 M+H(^+) (exact mass: 2893.70)</td>
</tr>
</tbody>
</table>
B. - CD Spectroscopy
All spectra were measured on a Jasco J-810 spectropolarimeter at peptide concentrations of 6 mg/mL in 100 mM sodium phosphate buffer at pH 7.4 and 20°C. A high peptide concentration was chosen, because at lower concentrations depsipeptides only rarely folded and had only a partially α-helical structure. Small variations in the peptide concentrations did not change the CD-spectra or the denaturation curves. The mean residue molar ellipticity was calculated using the following equation:

\[ \Theta = \left( \Theta_{\text{obs}} \times MRM \right) / (10 \times l \times c) \]

Where \( \Theta_{\text{obs}} \) is the observed ellipticity expressed in mdeg, \( MRM \) is the total molecular mass (TFA-salt) of the peptide divided by the number of amino acid residues, \( l \) is the optical path length in cm (0.01 cm), and \( c \) is the final peptide concentration in mg/mL.

C. - Chemical denaturation experiments
For all chemical denaturation experiments, the change in the ellipticity was followed at 222 nm, which is indicative for the helical content of peptides. An equilibration time of 3 h was used. Increasing the equilibration time above 3 h did not change the curve. GuHCl was purchased from Fluka. Concentration of the GuHCl stock solution was determined by the refractive index. The denaturation curves were fitted by using the program Sigma Plot 10.0 (sigmoidal - 5 parameter; exponential rise to max. - double 5 parameter).

**Thermodynamic Analysis** \(^4, \, 1, \, 5\)

\[
N \leftrightarrow U \\
f_N = (\Theta - \Theta_U) / (\Theta_N - \Theta_U) \\
K_U = f_U / f_N = f_U / (1 - f_U) \\
\Delta G_U = -RT \cdot \ln(K) \\
\Delta G_U = -RT \cdot \ln(f_U / (1 - f_U))
\]

The linear dependence of \( \Delta G_U \) on denaturant concentration observed in the transition region continues to zero concentration and fit data to an equation of the form \(^6\):

\[
\Delta G_U = \Delta G_U^{\text{H}_2\text{O}} - m [\text{GuHCl}] \\
\Delta G_U^{\text{H}_2\text{O}} = \Delta G_U + m [\text{GuHCl}] \\
\Delta G_U^{\text{H}_2\text{O}} = -RT \cdot \ln(f_U / 1 - f_U) + m [\text{GuHCl}] \\
\text{At } D_{50}\% \text{ follows } f_U = 1 - f_U \quad \Rightarrow \quad \Delta G_U^{\text{H}_2\text{O}} = m [\text{GuHCl} (D_{50}\%)]
\]
Calculation of the $\Delta \Delta G_{\text{H}_2\text{O}}$ values:

$$
\Delta \Delta G_{\text{H}_2\text{O}}^{(2)} = \left[ \frac{m_{(\text{pp})} + m_{(2)}}{2} \right] \times \left[ D_{50\%\text{ (2)}} - D_{50\%\text{ (pp)}} \right]
$$

D. - Size Exclusion Chromatography

Size exclusion chromatography of the parent peptide (pp) was accomplished with an Äkta FPLC system equipped with a Superdex 30 column (HiLoad 16/60 prep grade) from Amersham Biosciences. Elution buffer was 10 mM sodium phosphate pH 7.4 and the flowrate was 1.2 mL/min. Peptide absorbance of 0.5 mg pp was registered at 254 nm.

Figure S1: Size exclusion chromatogram of the pp.
E. – *Molecular Dynamics Simulations*

The MD-simulations were carried out with the Gromacs suite\(^8\) using the Gromos 53a6 force field.\(^9\) A twin range cut-off for van der Waals (0.9/1.4 nm) and a smooth particle mesh Ewald algorithm for Coulomb interactions (switching distance of 0.9 nm) were used. The neighbor lists were updated every 0.01 ps.\(^10\) Temperature (320 K) and pressure (1 bar) were kept constant by Berendsen weak coupling, with coupling constants of 0.1 ps for the temperature and 1 ps for the pressure.\(^11\) Constraints were applied to the bonds of the peptide with the LINCS algorithm.\(^12\) The coiled coil dimers were solvated in dodecahedric boxes with around 9,500 SPC water molecules, periodic boundary conditions were applied.

The simulations were performed on servers with 2 Opteron Dual Core processors and a clockspeed of 2.2 GHz; each ns of simulation required about 9 h of computation time.

To ensure the validity of the MD simulations the trajectory of pp was recalculated with different initial velocities. The shown trajectory pp_run1 is the one shown in the paper in Figure 2b, pp_run2 was recalculated. One clearly sees the, to a certain level, random based nature of MD simulations. Nevertheless, it is clearly shown that both simulations correlate very well.