Effects of a disulfide bridge on the helix in C-peptide analogs

Eunice J. York[a], John M. Stewart[a], Robert Fairman[b] and Robert L. Baldwin[b]
[a]Department of Biochemistry, University of Colorado School of Medicine, Denver, CO 80262, U.S.A.
[b]Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305, U.S.A.

Introduction

Modifications of the C-peptide of RNase A (residues 1–13) have led to analogs of increased helicity as measured by CD at 222 nm [1]. The reference peptides RN-21 (Ac-A-E-T-A-A-A-K-F-L-R-A-H-A-NH_2) and RN-80 (RN-21 : $Phe^8 \rightarrow$ Tyr) are about 50% helical in H_2O (pH 5.3, 3°C, 0.1 M NaCl). Introduction of an intramolecular disulfide bond across one turn of the helix might impart increased stability to these structures; computer modeling indicated that substitution of homocysteine (Hcy) in positions 7 and 11 of RN-21 with formation of a disulfide bond would not distort the helix and would not interfere with either the Glu^{2-}...Arg^{10+} ion pair or the Phe^8...His^{12+} interaction.

RN-83 (Ac-A-E-T-A-A-A-Hcy-F-L-R-Hcy-H-A-NH_2) was synthesized, and the bridge was formed both by oxidation of the free SH peptide and by use of the nitrophenylsulfenyl (Npys) group for disulfide-bond formation [2].

Results and Discussion

Synthesis was performed by SPPS methods using Boc chemistry; peptides were purified and characterized by standard methods [3]. Boc-Hcy(MeB) (Chemical Dynamics) was a gift from Peter S. Kim (MIT), and Npys-Cl was prepared by Wieslaw Klis in our laboratory. In the N-terminal region 1–6, 0.4 M KSCN was used in the coupling mixture in order to disrupt secondary structure of the growing peptide resin and increase the coupling rate [4]. Cleavage of Hcy(MeB) peptides employed a low-high HF method using anisole as the scavenger [5]. The free SH peptide, synthesized with the HF-labile MeB protection on Hcy, was reduced with DTT prior to air oxidation or was oxidized with $K_3Fe(CN)_6$. Alternately, use of the HF-stable Npys group for protection of Hcy made possible the purification of the peptide before formation of the disulfide bond. Npys was introduced by displacement of MeB on Hcy by Npys-Cl in the completed peptide resin. Formation of the disulfide bond proceeded by reaction of triphenylphosphine with Npys, giving either one free SH that could displace the second Npys with concomitant formation of the S—S bond, or two free SH groups

with S—S bond formation, by air oxidation. Subsequently, RN-92 (RN-83: Phe → Tyr) was synthesized in order to use the tyrosine absorbance as a measure of peptide concentration for CD measurements. CD was performed as previously described [1] on an AVIV 60DS spectropolarimeter.

The methods used to form the disulfide bond in RN-83 all gave an identical major product, albeit in low yield, that was ascertained to be the desired product by AAA and FABMS, RN-92 was synthesized by the air oxidation method. The CD of RN-92 shows a higher helix content (θ_{222} = −15 800, pH 5.3, 3°C, 0.1 M NaCl) than the reference peptide RN-80 (−12 700). Partial reduction of RN-92 causes a sharp decrease to −12 400. It is not yet clear if RN-92 is purely an α-helical structure in the oxidized form. The 222-nm minimum is shifted slightly to lower wavelength vs. the reduced peptide. Although the temperature dependence of θ_{222} shows a high T_m of 32°C, indicating a highly stable structure (the T_m for RN-80 is 0–5°C), it extrapolates to −19 000, lower than the expected −27 000. TFE titration gives a maximum θ_{222} of −19 000 at 12.5 mol% TFE, consistent with the temperature dependence data, whereas the reduced form (which is probably not fully reduced as shown by the presence of S—S bonds in the 260–280 nm region of the CD spectrum) is as high as −22 000. Also, the difference spectrum of the oxidized and reduced forms shows a miminum between 211 and 215 nm, which is not typical of an α-helix. The disulfide bond may have produced a kink or a twist in the backbone, and further experiments will be needed to characterize fully the secondary structure of these peptides.

Acknowledgements

This research was supported by NIH Grant GM-31475 to R.L.B. We thank Thanh Le for AAA and Leland V. Miller of the NIH Clinical Mass Spectrometry Resource, University of Colorado, supported by Grant RR-01152, for the FABMS. E.J.Y. thanks Applied Biosystems Inc. for a travel grant.

References

1. Shoemaker, K.R., Kim, P.S., York, E.J., Stewart, J.M. and Baldwin, R.L, Nature, 326 (1987) 563.
2. Bernatowicz, M.S., Matsueda, R. and Matsueda, G.R., Int. J. Pept. Prot. Res., 28 (1986) 107.
3. Stewart, J.M. and Young, J.D., Solid Phase Peptide Synthesis, 2nd edn. Pierce Chemical Comp., Rockford, IL, 1984.
4. Klis, W. and Stewart, J.M., In Rivier, J.E. and Marshall, G.R. (Eds.) Peptides: Chemistry, Structure and Biology (Proceedings of the 11th American Peptide Symposium), ESCOM, Leiden, 1990, p. 904.
5. Ploux, O., Chassaing, G. and Marquet, A., Int. J. Pept. Prot. Res., 29 (1987) 162.