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Polydepsipeptides. A systematic investigation of guest-host effects‡

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PROLOGUE

This paper should have been written 20 years ago. When I left Murray Goodman's laboratory, in 1985, after 3 years of post-doctoral work, I had not finished this project. Resolved to complete it once back in Padova, I carried out some of the computations, but I never felt that the job was done. I realize now that I did Murray a disservice, and I would like to make up for it with this contribution.

The idea behind this project is typical of how Murray worked, and is an example of what I learned from him: Murray took pleasure in understanding the relationship between chemical modifications and the properties of the molecules he created, whether physical, chemical or biological. He sought this understanding thoroughly and methodically, studying series upon series of compounds. In his laboratory, I learned to appreciate this systematic approach. The faults and limits of what I report here are mine, the ingenuity of the approach is his.

INTRODUCTION

Depsipeptides are peptides in which some of the amide linkages are replaced by ester bonds. The replacement is almost isosteric and the dipolar characteristics are also similar, leading to similar conformational preferences. However, the ester group cannot be a donor in a hydrogen bond and can therefore be used to investigate the relative importance of this stabilizing interaction on the conformation of peptides. Murray developed this theme starting in the early 1970s, utilizing spectroscopic tools such as CD, IR absorption and NMR, as well as theoretical calculations, applied to both polydepsipeptides and related model compounds.

The work presented here is an attempt to distinguish two contributions to the CD spectrum of a depsipeptide: the conformational effects induced by the replacement of the amide with an ester, and the intrinsic changes caused by the different chromophore. A series of *tert*-butyloxycarbonyl (Boc)-protected oligoglutamates with the general formula Boc-[L-Glu(OMe)]$_n$-OMe (OMe, methoxy) ($n = 2$–7) was modified by substituting one Glu(OMe) residue with a lactic acid (Lac) moiety. The CD spectrum of each compound was recorded in TFE at a concentration of approximately 10^{-3} mol/l, and various difference spectra were analysed by spectral deconvolution. A preliminary account of this work appeared in the proceedings of an international symposium [1].

In a landmark study, Murray had applied CD and NMR 'to the problem of the critical size for helix formation in oligopeptides' [2]. Investigating a series of [L-Glu(OEt)]$_n$ (OEt, ethoxy) oligopeptides, he concluded that both techniques 'clearly indicate the onset of helicity at about the heptamer for these peptides in solvents such as trifluoroethanol and trimethylphosphate' [2]. Although [L-Glu(OEt)]$_n$ oligopeptides shorter than seven residues adopt a completely random conformation and the ellipticity at 222 nm is negligible in their CD spectra, these spectra are different from one another [2], indicating important contributions of main-chain end effects. Bayley, Nielsen and Schellman [3] also showed that the two amide groups in a dipeptide contribute differently to the CD spectrum. Subtraction of the CD spectrum of Boc-[L-Glu(OMe)]$_{n-1}$-OMe from that of Boc-[L-Glu(OMe)]$_n$-OMe should eliminate end effects and reveal the intrinsic contribution of just one chromophore if the conformation of the two peptides is the same. This approach was utilized by Toniolo and Bonora [4] to evaluate the contribution of internal peptide chromophores to the CD spectrum by subtracting the spectra of homo-trimers from those of homo-tetramers. Similarly, subtraction of the CD spectrum of a depsipeptide in which one Lac residue replaces one Glu(OMe) in the series Boc-[L-Glu(OMe)]$_n$-OMe ($n = 3$–7) from that of the parent peptide should indicate the intrinsic effect of an amide-to-ester substitution.

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§ Murray Goodman passed away on 1 June 2004.
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The observable electronic transitions in peptides are the amide n-π^* at 224 nm and the π-π^* at 184 nm [5]. In depsipeptides, the ester n-π^* transition occurs at 213 nm and the π-π^* transition at 167 nm [6]. Three mechanisms, reviewed by Schellman and coworkers [3,7], can give rise to optical activity in these transitions: the one-electron mechanism, the Kirkwood-Moffitt mechanism of dipole coupling, and the electric-magnetic, or μ-m, coupling.

BOC-(L-GLU(OME))$_n$-OME SERIES

The difference spectra are shown in Figure 1 and the parameters obtained from the fitting are reported in Table 1. In the simplest case ($n = 3$), a good fit is obtained with two Gaussian curves, one at 196 nm and one at 218 nm. The n-π^* transition is very weak and no exciton splitting seems to be present in the π-π^* transition, probably due to conformational averaging which prevents coupling between transitions of two different chromophores.

To fit the difference spectra for $n = 4$ and $n = 5$, two Gaussian curves of equal intensity and opposite sign are necessary in the π-π^* region, indicating that the dipole coupling mechanism is effective already at the level of the tetramer. In both cases, the n-π^* transition is very weak, suggesting that no μ-m coupling is present. The much bigger change induced by the insertion of the fourth Glu(OMe) residue relative to the insertion of the fifth seems to imply that the conformations allowed and preferred by the tetramer and the pentamer are relatively similar.

The difference spectrum for $n = 6$ shows some new features: the n-π^* band is more negative than in any other spectrum. Moreover, the positive component of the exciton splitting is above 190 nm and its intensity is slightly different from that of the negative one. It is possible that helical conformations are visited by the hexamer to an appreciable extent. The helix is definitely nucleated by the insertion of the last Glu(OMe) residue. The relevant difference spectrum ($n = 7$) is fitted by a very intense positive band at

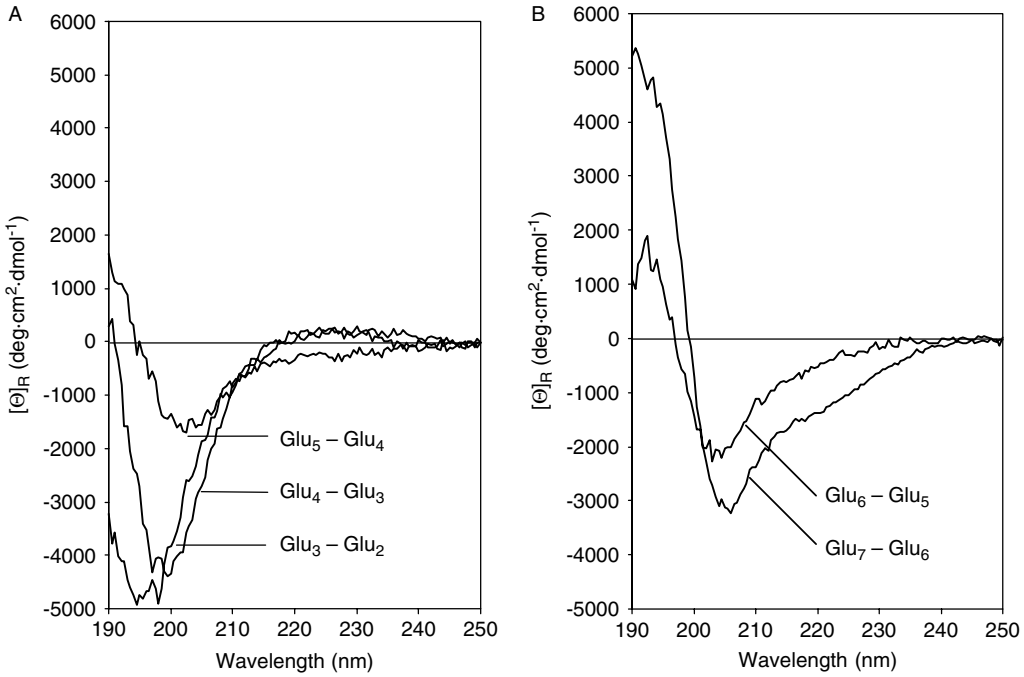

Figure 1 Difference CD spectra Boc-[Glu(OMe)]$_n$-OMe — Boc-[Glu(OMe)]$_{n-1}$-OMe in TFE. (A) $n = 3$–5. (B) $n = 6$–7.

Table 1 Best-fit Parameters for the Difference CD Spectra Boc-[Glu(OMe)]$_n$-OMe — Boc-[Glu(OMe)]$_{n-1}$-OMe in TFE

Spectrum	λ_1 (nm)	Rel. Int.	Width (nm)	λ_2 (nm)	Rel. Int.	Width (nm)	λ_3 (nm)	Rel. Int.	Width (nm)
Glu_3—Glu_2	—	—	—	196	−0.805	16	218	−0.096	25
Glu_4—Glu_3	184	0.796	16	198	−0.796	16	222	0.040	25
Glu_5—Glu_4	188	0.259	14	202	−0.259	14	231	0.041	20
Glu_6—Glu_5	196	0.669	14	200	−0.741	14	218	−0.111	20
Glu_7—Glu_6	193	0.918	14	202	−0.604	14	219	−0.287	20

193 nm and a less intense negative one at 202 nm, while the n-π^* band at 219 nm is negative and more intense than in previous cases. These results are consistent with the onset, already at the level of the hexamer, of coupling between the electric moment of the π-π^* transition with the magnetic moment of the n-π^* transition of a different peptide group. The low intensity ratio between the two negative bands around 220 and 200 nm could be interpreted by the presence of the 3_{10}-helix [8]. However, the present data do not allow us to distinguish between α- and 3_{10}-helices. Indeed, the low intensity of the n-π^* transition could arise either from the less-than-optimal disposition of successive peptide groups in a 3_{10}-helical arrangement or from a small residence time of the hexamer and the heptamer in the (α)-helical conformation.

COMPARISON WITH DEPSIPEPTIDES

The oligoglutamates described in the preceding section were compared with their analogues in which one internal residue was replaced by a Lac moiety. Although the difference spectra should contain contributions from four transitions (the π-π^* and the n-π^* of the amide that was replaced and the π-π^* and the n-π^* of the ester that was introduced), only two transitions are visible, because the π-π^* of the ester occurs at too high an energy and the n-π^* of the amide is very weak, at least until the hexamer. The optical activity of the n-π^* transition of the ester arises from coupling of its magnetic moment with the electric moment of the π-π^* transition of the amide. This mechanism should be more effective for ester–amide interaction, because the transitions involved are closer in energy than in the corresponding amide–amide interaction.

Trimers

The difference CD curve [Glu_3 — Glu-Lac-Glu] (Figure 2) cannot be fitted with two gaussians. The π-π^* transition of the amide requires a positive contribution at lower wavelengths that can be accounted for as an exciton splitting curve (Table 2). The substitution of the amide with the ester appears to disrupt a dipole coupling present in Boc-[Glu(OMe)]$_3$-OMe, while we had concluded from the difference curve between the trimer and the dimer that no coupling is present between the two internal amides. These two results indicate that the dipole coupling disrupted by the ester is the one between the urethane protecting group and the first amide.

Tetramers

In both difference spectra, the fitting is possible with an exciton splitting for the π-π^* transition and a third gaussian for the n-π^* transition. The exciton curves for [Glu_4 — Glu-Lac-Glu_2] are similar to those for [Glu_3 — Glu-Lac-Glu], indicating a similar effect of the ester. In the case of [Glu_4 — Glu_2-Lac-Glu], the fitted exciton curves are much more intense, as expected for the disruption of dipole coupling between two consecutive amides.

Pentamers

A Lac residue at position 2 affects the CD spectrum much less than in the other two cases, confirming the results from the tetramers: the smallest effect is found when the highest number of consecutive amides is left untouched. As expected, the difference curves for Lac at position 3 or 4 are very similar since the same couplings are possible in both cases.

Table 2 Best-fit Parameters for the Difference CD Spectra Boc-[Glu(OMe)]$_n$-OMe — Boc-[Glu(OMe)]$_m$-Lac-[Glu(OMe)]$_{n-m-1}$-OMe in TFE

Spectrum	λ_1 (nm)	Rel. Int.	Width (nm)	λ_2 (nm)	Rel. Int.	Width (nm)	λ_3 (nm)	Rel. Int.	Width (nm)	λ_4 (nm)	Rel. Int.	Width (nm)
Glu_3—Glu-Lac-Glu	182	1.141	16	196	−1.141	16	216	−0.682	25			
Glu_4—Glu-Lac-Glu_2	182	1.011	16	198	−1.011	16	218	−0.192	25			
Glu_4—Glu_2-Lac-Glu	184	1.867	16	197	−1.867	16	214	−0.328	25			
Glu_5—Glu-Lac-Glu_3	186	0.758	16	200	−0.481	16	221	−0.025	20			
Glu_5—Glu_2-Lac-Glu_2	188	1.755	16	197	−1.820	16	209	−0.156	20			
Glu_5—Glu_3-Lac-Glu	184	1.792	16	199	−1.532	16	216	−0.101	20			
Glu_6—Glu-Lac-Glu_4	194	0.533	14	200	−0.394	14	211	−0.161	20			
Glu_6—Glu_2-Lac-Glu_3	195	0.801	14	199	−0.801	14	215	−0.032	20	229	0.046	20
Glu_6—Glu_3-Lac-Glu_2	191	1.351	14	199	1.213	14	208	−0.319	20	228	0.054	20
Glu_7—Glu-Lac-Glu_5	196	1.989	14	199	−1.701	14	214	−0.211	20			
Glu_7—Glu_2-Lac-Glu_4	193	0.614	14	201	−0.762	14	226	0.126	20			
Glu_7—Glu_3-Lac-Glu_3	192	1.357	14	202	1.300	14	217	−0.264	20			

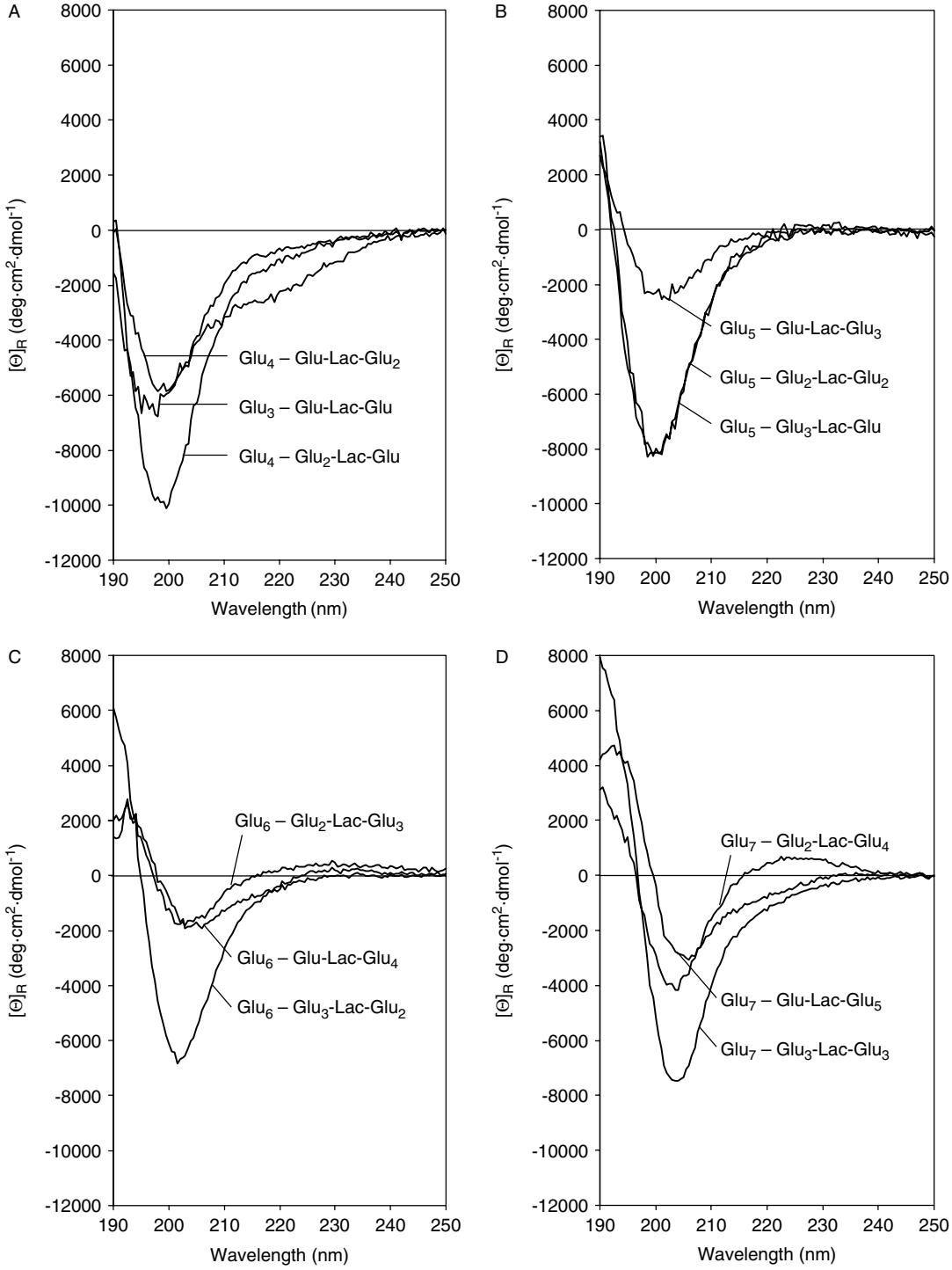

Figure 2 Difference CD spectra Boc-[Glu(OMe)]$_n$-OMe — Boc-[Glu(OMe)]$_m$-Lac-[Glu(OMe)]$_{n-m-1}$-OMe in TFE. (A) $n = 3$–4. (B) $n = 5$. (C) $n = 6$. (D) $n = 7$.

Hexamers

The effect of Lac either at position 2 or 3 is similar and very small, as a high number of consecutive amides are present in both cases. A much bigger disruption of dipole coupling is seen when Lac is at position 4. The difference curves for substitution at positions 3 and 4 cannot be fitted with only three gaussians: a fourth one is needed, although at a very weak intensity. Possibly, this curve accounts for the n-π^* transition of the amide, present for the first time.

Heptamers

Three depsipeptides in this series were available to us. Only in the case of Lac at position 4, could the exchange

of an amide for an ester interfere with the formation of an α-helical H-bond. In fact, the difference CD spectrum for [Glu_7 — Glu_3-Lac-Glu_3] is the most intense and the fitted contribution in the n-π^* region is significant.

CONCLUSIONS

The fitting of the difference CD curves in the Boc-[L-Glu(OMe)]$_n$-OMe series clearly indicates that even though the conformation of all the peptides is considered to be random, the actual conformations visited by the different oligomers are not the same, as demonstrated by the different contribution of the dipole coupling. A much bigger change is seen going from the trimer to the tetramer than from the tetramer to the pentamer, suggesting that three consecutive amide groups are critical for the development of sizable dipole coupling effects. The difference spectrum [Glu_6 — Glu_5] clearly indicates that even the hexapeptide probes α-helical conformations.

The intrinsic effect of the ester substitution on the CD spectrum of a random coiled peptide is evident from this study: a weak contribution in the n-π^* region and a definite disruption of amide π-π^* dipole couplings. The next step would be to apply this approach to polymers in which both an intrinsic and a conformational effect of the ester are possible.

FINAL REMARKS

The work presented here lacks the scientific impact it could have had 20 years ago. The field has progressed much beyond the scope of this work. And yet, the foundation for today's research in the areas of peptide and protein conformation and dynamics was laid by studying model compounds by all available means. We owe much to the pioneers in this type of research, a brilliant example of whom was Murray Goodman.

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