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Effect of temperature on peptide chain aggregation: an EPR study of model peptidyl-resins

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Abstract—The effect of temperature on the dynamics of peptide chains inside resin beads was monitored by electron paramagnetic resonance (EPR) spectroscopy. A two-component spectra was obtained for low and highly peptide-loaded model peptidyl-resins labeled with the paramagnetic aminoacid 2,2,6,6-tetramethyl-piperidine-*N*-oxyl-4-amino-4-carboxylic acid (TOAC), indicating the presence of strongly and weakly immobilized populations. Increasing levels of chain disaggregation were observed with increasing temperature, leading in some cases to a complete disappearance of the more immobilized population. The present findings demonstrate that EPR spectral parameters are highly sensitive to the solvation properties of labeled sites inside the resin matrix and can be of great value for the understanding of polymer-supported processes or reactions. © 2001 Elsevier Science Ltd. All rights reserved.

Merrifield's solid-phase peptide synthesis method[1] has been intensively investigated as a convenient model to improve the knowledge of the physicochemical basis of processes occurring throughout a polymeric matrix. In this context, emphasis has been recently given to resin-supported combinatorial chemistry for the development of new drugs.[2,3] Despite all these continuous efforts to optimize the peptide synthesis protocol, serious shortcomings have intriguingly persisted. They are mainly related to incomplete coupling during peptide growth caused by chain aggregation inside the bead.[4] To overcome this problem alternative solid supports have been continuously proposed for peptide synthesis.[5,6] In addition, attempts to establish the rules which govern resin solvation have been the object of several publications, as this property has been found to depend upon the

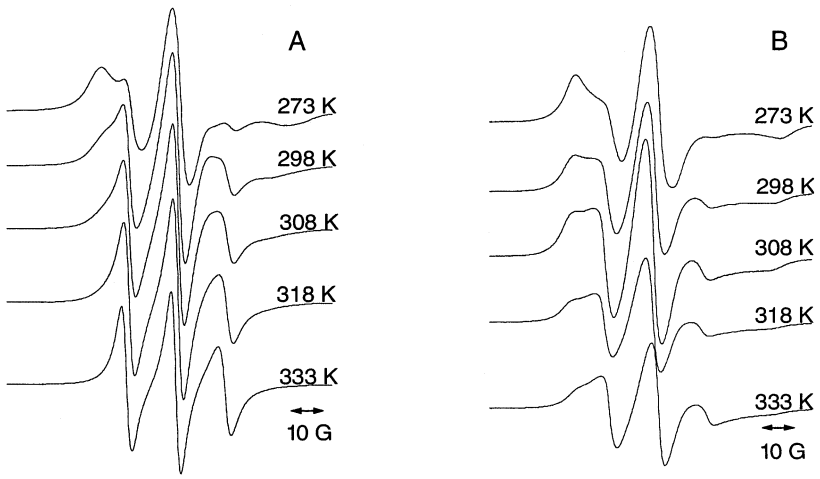

Figure 1. Effect of temperature upon the EPR spectra of TOAC-labeled low (A) and highly loaded (B) VQAAIDYING-BHAR in DMF.

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resin, peptide sequence and loading, as well as the solvent system.[7,8] We and other groups have applied EPR spectroscopy to investigate the solvation properties of peptidyl-resins.[9–11] The goal of the present report is to extend this strategy to examine the effect of temperature on the dynamics of model peptidyl-resin beads and its possible implications in the peptide synthesis methodology.

The VQAAIDYING (65–74) fragment of the acyl carrier protein[12] and the (CRF^{34-41}) corticotrophin releasing factor antagonist [NRKL(Nleu)EII][13] were synthesized through the conventional Boc/Bzl-solid-phase method[14] using benzhydryl- and methylbenzhydrylamine-resins (BHAR and MBHAR, respectively), in low and highly substituted conditions (up to 3.0 mmol/g). The latter conditions were designed to promote chain association inside the resin beads. For EPR studies, similarly to the labeling strategy used for free peptides, the peptidyl-resins were labeled with the *N*-protected[15,16] paramagnetic aminoacid 2,2,6,6-tetramethylpiperidine-*N*-oxyl-4-amino-4-carboxylic acid (TOAC). In order to avoid spin–spin exchange interactions, which may broaden the EPR lines and to minimize possible physicochemical and steric perturbations, the extent of labeling was kept as low as possible. It was also assumed that the TOAC-labeled peptide chains are dispersed homogeneously throughout the resin matrix and behave similarly to the unlabeled chains in all solvent systems tested. Samples were placed in flat quartz cells and EPR measurements were carried out at 9.5 GHz in a Bruker ER 200 spectrometer using a variable-temperature accessory. The temperature was monitored making use of a thermocouple. Labeled peptidyl-resin beads were pre-swollen in the solvent under study for 24 h. The samples were equilibrated at the desired temperature for approximately 5 min before running the spectra. The magnetic field was modulated with amplitudes less than one-fifth of the linewidths, and the microwave power was 5 mW to avoid saturation effects.

Fig. 1 displays the EPR spectra of low and highly peptide-loaded (30 and 83% peptide content, respectively) VQAAIDYING-BHAR swollen in dimethylformamide (DMF) in the 273–333 K temperature range. In most spectra two components are present: a broad one and a narrow one, due to strongly and weakly immobilized populations, respectively. As expected, the motion increased with increasing temperature, as indicated by the narrowing of the spectral lines correspond-

Table 1. Effect of temperature on the EPR spectra of low and highly loaded TOAC-labeled VQAAIDYING-BHAR swollen in DMF

Temperature (K)	BHAR (degree of substitution)					
	0.3 mmol/g			3.0 mmol/g		
	W_0 (G)	h_{-1}/h_0	A_{max} (G)[a]	W_0 (G)	h_{-1}/h_0	A_{max} (G)[a]
273	5.50	0.008	57.0	6.98	0.026	63.4
298	4.17	0.184	Nd	5.50	0.063	63.3
308	3.57	0.258	Nd	5.10	0.092	61.0
318	3.27	0.341	Nd	4.76	0.123	60.0
333	3.00	0.484	Nd	4.20	0.165	Nd

[a] Nd, not determined.

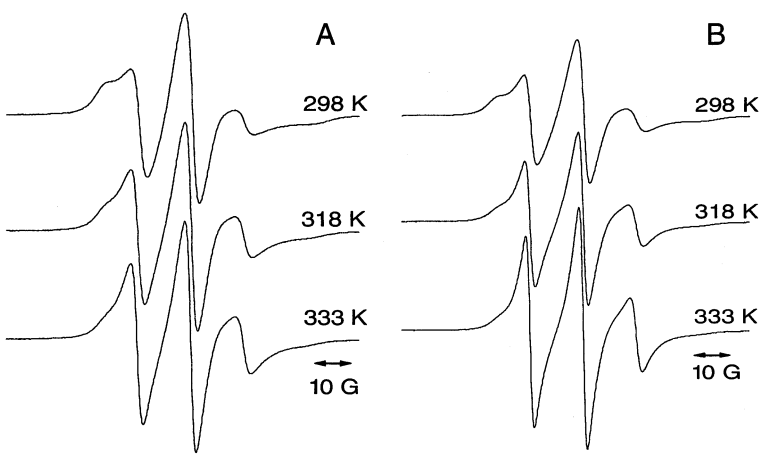

Figure 2. Effect of temperature on the EPR spectra of TOAC-labeled highly loaded NRKL(Nleu)EII-MBHAR in DMF (A) and in DMSO (B).

Table 2. Effect of temperature on the EPR spectra of highly loaded TOAC-labeled NRKL(Nleu)EII-MBHAR swollen in DMF and DMSO.

Temperature (K)	Solvent					
	DMF			DMSO		
	W_0 (G)	h_{-1}/h_0	A_{max} (G)[a]	W_0 (G)	h_{-1}/h_0	A_{max} (G)[a]
298	4.21	0.13	58.0	3.28	0.16	Nd
318	3.72	0.20	Nd	2.91	0.24	Nd
333	3.42	0.25	Nd	2.49	0.30	Nd

[a] Nd, not determined.

ing to both populations. A clear difference in the solvation properties of the two resins is observed with increasing temperature. It can be noted that the heavily peptide-loaded resin presents a larger contribution of the more immobilized population (Fig. 1B), very likely as a consequence of a higher degree of chain association. In contrast, a progressive disappearance of the more immobilized component was observed with the low peptide-loaded resin (Fig. 1A). This component became essentially undetectable at 333 K. Table 1 presents the values of some EPR spectral parameters whose values can be correlated with the dynamics of labeled sites in the resin. We measured the central field peak linewidth (W_0), that contains the contribution of both populations, the ratio of heights of the high and mid-field lines (h_{-1}/h_0), where h_{-1} corresponds essentially to the weakly immobilized component and the separation between the outer extrema (A_{max}), that corresponds essentially to the more immobilized component. The lower the values of W_0 and A_{max} and the higher the h_{-1}/h_0 ratio, the faster the motion of the labeled sites. Accordingly, a significant decrease in W_0 and A_{max} and an increase in h_{-1}/h_0 are observed as the temperature increases from 273 to 333 K. Table 1 also corroborates the differences between the low and highly peptide-loaded resins, emphasizing the challenge involved in synthesizing peptides in heavily chain loaded conditions.

Fig. 2 shows the EPR spectra of highly loaded NRKL(Nleu)EII-MBHAR (78% peptide content) swollen in DMF and in dimethylsulfoxide (DMSO) at different temperatures. A small, yet significant increase in chain mobility occurs in the latter solvent, as indicated by the greater contribution of the more mobile component in Fig. 2B and by EPR parameters shown in Table 2.

These results indicate that the latter solvent is more appropriate for the synthesis of this sequence, in heavily loaded conditions. Furthermore, the larger W_0 values at higher loading in the spectra of VQAAIDYING (Table 1) when compared to NRKL(Nleu)EII (Table 1), regardless of temperature, suggest that the former sequence is characterized by a more pronounced tendency to self-associate.

Taken together, the present findings point to the usefulness of increasing the temperature to improve the peptide synthesis yield, as already discussed.[17] However, care should be taken, since this effect seems to depend on several parameters, including the sequence to be assembled.[18,19] Nevertheless, the evaluation of the effect of temperature on the dynamics of peptidyl-resins demonstrates that EPR can be used as a very sensitive tool to discriminate the structural characteristics of each specific sequence even inside very complex and heterogeneous media. Moreover, due to the spin probe location in the peptide sequence, the present approach is unique for the detection of different interchain association levels at the N-terminal region, where the extent of steric hindrance is critical for the success of the synthesis.

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