Letters

Influence of molecular weight on the tensile properties of nearly monodisperse polystyrenes

There have been relatively few studies of the influence of molecular weight upon the tensile properties of glassy polymers[1]. It is generally accepted, however, that both tensile strength and rupture elongation increase as the molecular weight increases up to a certain value and remain nearly constant thereafter. Some results on polystyrene have been reported by Merz *et al.*[2], McCormick *et al.*[3], Wyman *et al.*[4] and by Thomas and Hagan[5]. From the excellent review paper of Martin *et al.*[1] it can be gathered that in many instances the influence of molecular weight has been confused with that of the molecular weight distribution. In the present investigation nearly monodisperse polystyrenes were used, purchased from Pressure Chemical Co., Pittsburgh, Pa.

The test specimens were made by Maxwell's[6] miniature mixing and injection moulding machine manufactured by Custom Scientific Instruments, Inc., Whippany, NJ. These specimens were very small (about 0.2 g) and in a usual 'dumb-bell' shape with an overall length of 3/4 in. and a 1/16 in. diameter by 5/16 in. long test section. The same standard procedure was followed for the preparation of all the specimens before testing. Moulding temperatures were in the range of 170°–200°C and after a mixing time of about 2 min the specimens were allowed to cool in air at room temperature.

Table 1 Tensile properties of nearly monodisperse polystyrenes at room temperature. Elongation rate: 1.5×10^{-3} sec^{-1}

$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	Tensile strength (kPa)	Rupture elongation (%)
51 000	<1.06	18 750	
97 200	<1.06	26 200	2.48
200 000	<1.06	46 200	4.70
411 000	<1.15	51 020	5.62
498 000	<1.20	52 920	6.37
670 000	<1.15	49 640	5.97
860 000	<1.15	50 050	5.66
2 000 000	<1.20	54 670	5.87

The measurements were performed on a Mini Max tensile tester also made by Custom Scientific Instruments, Inc. Fifteen specimens from each polystyrene sample were tested and the results are shown in *Table 1*. Deviations as high as 15% from the average values given were observed in certain samples.

From *Table 1* it can be gathered that the tensile strength increases with molecular weight up to a value of $\bar{M}_w$ between 300 000 and 400 000. This result is fully in agreement with Bondurant's data[7] which show that the tensile strength of polystyrene fractions does not become constant until $\bar{M}_w$ = 350 000. Our results show that the rupture elongation also increases with molecular weight and becomes constant at about M_w = 400 000. It should also be noted that, except for the samples of the two lower molecular weights, the specimens exhibited a 'yield point' before rupture.

Acknowledgement

Financial assistance from the National Research Council of Canada is gratefully acknowledged.

References

1 Martin, J. R., Johnson, J. F. and Cooper, A. R. *J. Macromol. Sci. (C)* 1972, **8**, 57
2 Merz, E. H., Nielsen, L. E. and Buchdahl, R. *Ind. Eng. Chem.* 1951, **43**, 1396
3 McCormick, H. W., Brower, F. M. and Kin, L. *J. Polym. Sci.* 1959, **39**, 87
4 Wyman, D. P., Elyash, L. J. and Frazer, W. J. *J. Polym. Sci. (A)* 1965, **3**, 681
5 Thomas, D. P. and Hagan, R. S. *Polym. Eng. Sci.* 1969, **9**, 164
6 Maxwell, B. *SPE J.* 1972, **28**, 24
7 Bondurant, C. W. *PhD Thesis* Virginia Polytechnic Institute (1960)

J. Vlachopoulos, N. Hadjis and A. E. Hamielec
Department of Chemical Engineering, McMaster University, Hamilton, Ontario L8S 4L7, Canada
(Received 28 August 1977; revised 12 September 1977)

Incorporation of spin probes into polynucleotides by enzymatic polymerization

Spin labelling is an effective tool for studying the structure–function relationships present in various complex biological systems. A variety of biological systems have been investigated by the spin label method, but nucleic acids thus far have received little attention. This is essentially due to the known difficulty of achieving site-specific chemical modifications of nucleic acids. For the past several years, one of us has used non-site-specifically spin labelled polynucleotides ('first generation spin labelled nucleic acids') for characterizing conformational transitions of nucleic acids, and nucleic acid–nucleic acid or nucleic acid–protein interactions[1]. The spin label experiments were carried out with probes tumbling in the 'motionally narrowing region'[2]. It was, therefore, hypothesized that it is not essential to achieve site specific labelling of nucleic acids, since the probe cannot be very sensitive to detailed structural features in that motion range: i.e., probes linked to different sites of the nucleic acid residue would give rise to similar e.s.r. spectra. However, perturbation of the probe region by formation of an inter–intramolecular association would cause e.s.r. spectra which are significantly different from those of unperturbed systems.

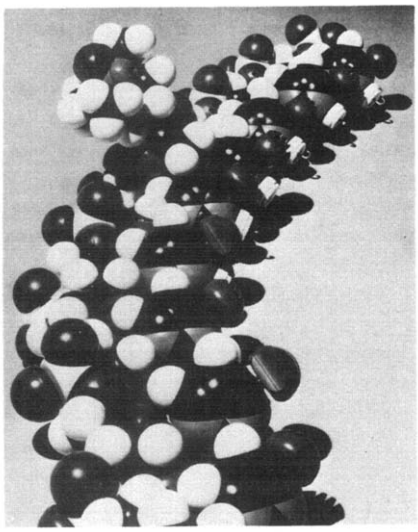

Figure 1 Molecular structure of (RUGT, U)$_n$: Ealing CPK atomic model showing a small stretch of the (RUGT, U)$_n$ chain (1 RUGT and 8U residues)

Here we wish to report the synthesis of a novel spin labelled nucleic acid which contains a random distribution of spin probes in site-specific position of the nucleic acid matrix. This was achieved by incorporating spin probes through enzymatic polymerization, and we believe that this is the first description of spin labelled nucleic acids or biopolymers obtained by such a route ('second generation spin labelled nucleic acids'). With a 'second generation spin labelled nucleic acid' it was also possible to confirm the above hypothesis.

Experimental

Synthesis of (RUGT,U)$_n$, a 'second generation spin labelled nucleic acid'. The 5-*O*-spin labelled derivative of 5-hydroxyuridine-5′-diphosphate, *N*-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]-*O*-[1-(5′-*O*-diphosphono-β-D-ribofuranosyl)-uracil-5-yl]-glycolamide (ppRU–Glycolamido–TEMPO = ppRUGT) was copolymerized with uridine-5′-diphosphate by the enzyme polynucleotide phosphorylase from *Micrococcus luteus* or *E. coli B.* ppRUGT was synthesized by alkylation of 5-hydroxyuridine 5′-diphosphate with 4-(α-chloroacetamido)-2,2,6,6-tetramethyl-piperidino-1-oxy in the presence of one equivalent of NaOH. Chromatography on Whatman 3MM paper with the solvent system ethanol–1M ammonium acetate (pH 7.5), 7:3 was used for the purification of ppRUGT. Digestion of ppRUGT by bacterial alkaline phosphatase gave as the only u.v. absorbing product *N*-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]-*O*-[1-β-D-ribofuranosyl-uracil-5-yl]-glycolamide (RU–Glycolamido–TEMPO = RUGT). The R_f of RUGT (silica gel t.l.c. with 16% methanol/chloroform) was 0.47. RUGT was also directly synthesized by 5-hydroxyuridine with 4-(α-chloroacetamido)-2,2,6,6-tetramethyl-piperidino-1-oxy in the presence of an equivalent NaOH. The structure of RUGT as well as the structure of the similarly prepared deoxy analogue, DUGT (DU–Glycolamido–TEMPO = *N*-[1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl]-*O*-[β-D-2′-deoxyribofuranosyluracil-5-yl]-glycolamide), were supported by elemental analysis and molecular weight (by electron impact mass spectrometry). Characterization of RUGT: m.p. (uncorrected) 131°–133°C; analysis calculated for $C_{20}H_{31}N_4O_9$: C 50.95, H 6.62, N 11.88; analysis found: C 50.97, H 7.25, N 11.71; Finnigan GC/MS: *m/e* 471 for M^+. Characterization of DUGT: m.p. (uncorrected) 121°–123°C; analysis calculated for $C_{20}H_{31}N_4O_8$: C 52.74, H 6.86, N 12.30; analysis found: C 52.29, H 7.18 N 12.30; Finnigan GC/MS: *m/e* 455 for M^+. In the meantime, the structure of the reduced form of RUGT and DUGT had also been corroborated by p.m.r.[3].

The weight-average degree of polymerization for (RUGT,U)$_n$ was calculated from the measured sedimentation coefficient, s_{20} 4,5. A weight-average molecular weight of 33 000 daltons for (RUGT,U)$_n$ was obtained in an incubation mixture containing per millilitre: 15 polymerization units of *E. coli B* polynucleotide phosphorylase, 0.1 mmol of Tris (pH 8.7), 10 μmol of uridine-5′-diphosphate, 0.75 μmol of ppRUGT and 2 μmol of $MnCl_2$. The reaction was carried out at 37°C and followed by P_i release. A weight-average molecular weight of 66 000 daltons for (RUGT,U)$_n$ was isolated by changing the polymerization procedure as follows: 15 polymerization units of *Micrococcus luteus* polynucleotide phosporylase were added to a reaction mixture containing 0.1 mmol of Tris (pH 8.7), 10 μmol of uridine-5′-diphosphate, 0.75 μmol of ppRUGT, 4 μmol of $MnCl_2$, and 0.6 mg bovine serum albumin. The reaction mixture was incubated at 46°C for 6 h and subsequently deproteinized several times by $CHCl_3$–isoamyl alcohol (5:2 v/v). Both the samples of 33 000 and 66 000 daltons (RUGT,U)$_u$ were purified by Sephadex G-100 chromatography before measurement of their physico-chemical properties. The RUGT/U ratio was determined to be 1/50 to 1/100 for both biopolymers.

Figure 1 shows an Ealing CPK atomic model of (RUGT,U)$_n$. It is apparent that the steric perturbation caused by incorporating a small extrinsic moiety in a long chain will likely have a minimal effect on most physico-chemical characteristics of the polymer. Earlier, it was shown with 'first generation spin labelled nucleic acids' that there is, in the case of spin labelled (A)$_n$·(U)$_n$ or spin labelled (U)$_n$·(A)$_n$, good agreement between T_m^{OD} (optical-density melting) and T_m^{SP} (spin melting)[6]. Thus, the overall physico-chemical properties of these biopolymers do not seem to be significantly perturbed upon introduction of the spin label, an important consideration when incorporating a foreign entity into a biological system.

Figure 2 shows the typical e.s.r. spectra of (RUGT,U)$_n$ in its single-stranded form (spectrum A) and when complexed with (A)$_n$ under conditions known to form a duplex[7] (spectrum B). The e.s.r. lineshapes are very similar to those reported earlier for duplexes consisting of (A)$_n$ and 'first generation spin labelled (U)$_n$ or (dUfl)$_n$'[6,8]. A detailed analysis of (RUGT,U)$_n$ containing e.s.r. spectra by computer simulation as done earlier for the 'first generation spin labelled nucleic acids'[8,9] is in progress.

As was reported earlier, spin labelled nucleic acids are an effective tool to determine relative affinities of nucleic acid binding proteins for various nucleic acids[10]. The e.s.r. spectra of (RUGT,U)$_n$ in the absence and presence of the unwinding gene 32 protein[11] (P32) are shown in *Figures 3A* and *3B*, respectively. As is clear from *Figure 3c*, the 10.5 nmol (RUGT,U)$_n$ in the (RUGT,U)$_n$–P32 complex are completely displaced upon addition of 10.6 nmol (dUfl)$_n$. This result corroborates the observation made previously with the 'first generation spin labelled (U)$_n$'. Previously, it was generally assumed that P32 is a 'non-specific site binding protein'[12], and

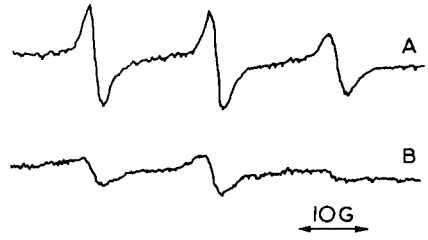

Figure 2 E.s.r. line shape change upon duplex formation between (RUGT, U)$_n$ and (A)$_n$ in 0.195 M NaCl, 0.01 M Tris, pH 7.5. A, 2.9 × 10^{-5} M (RUGT, U)$_n$; B, 2.9 × 10^{-5} M (RUGT, U)$_n$. (A)$_n$

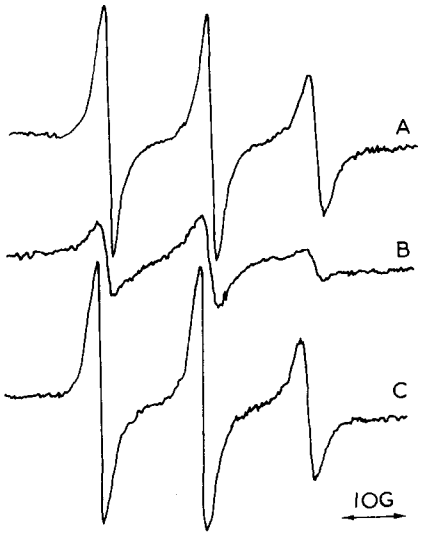

Figure 3 E.s.r. competition experiment with T4 gene 32 protein (P32) and (RUGT, U)$_n$ and (dUfl)$_n$. A, 10.5 nmol (RUGT, U)$_n$; B, (RUGT, U)$_n$–P32 complex [Nucleotide/P32 = 9.5, 10.5 nmol (RUGT, U)$_n$] C, addition of 10.6 nmol (dUfl)$_n$ to (RUGT, U)$_n$–P32 complex described in *Figure 3B*

therefore the displacement observations[10] were unexpected. In the meantime, it has been recognized that the standard error of the binding parameter measurements[13] could be large enough to give parameter differences for different polynucleotides which are large enough to explain the differences in the relative affinity of various polynucleotides for P32 as determined by e.s.r. competition experiments.

In conclusion, we report that spin probes can be incorporated by means of enzymatic synthesis into nucleic acids. Such labelled nucleic acids offer the possibility to study the structure–function relationships of nucleic acids in complex biological systems by e.s.r. This is especially likely since the snythesis described here may lead to the synthesis of a great variety of RUGT containing nucleic acids by copolymerizing ppRUGT not only with uridine-5′-diphosphate, but also with other nucleoside-5′-diphosphates.

Acknowledgement

One of us (A. M. B.) acknowledges support from the NIH Visiting Program and thanks Dr Nancy Nossal for discussions concerning the isolation of gene 32 protein and Dr B. Witkop for generous hospitality. The authors wish to thank Dr Leonard Kohn (NIH) for determination of the s_{20} values and Mr Michael Edwards for excellent technical assistance.

Albert M. Bobst* and Paul F. Torrence

Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, US National Institutes of Health, Bethseda, Maryland 20014, USA

(Received 17 August 1977)

References

1 Bobst, A. M. *Chimia* 1977, **31**, 141
2 Goldman, S. A., Bruno G. V., Polnaszek, C. F. and Freed, J. H. *J. Phys. Chem.* 1972, **76**, 716
3 Ozinskas, A. J. and Bobst, A. M. Manuscript in preparation
4 Inners, L. D. and Felsenfeld, G. *J. Mol. Biol.* 1970, **50**, 373
5 Janik, B. and Sommer, R. G. *Biopolymers* 1973, **12**, 2803
6 Pan, Y. E. and Bobst, A. M. *Biopolymers* 1973, **12**, 367
7 Blake, R. D., Massoulié, J. and Fresco, J. R. *J. Mol. Biol.* 1967, **30**, 291
8 Bobst, A. M., Sinha, T. K. and Pan, Y. C. E. *Science* 1975, **188**, 153
9 Pan, Y. E. *PhD Thesis* University of Cincinnati (1975)
10 Bobst, A. M. and Pan, Y. E. *Biochem. Biophys, Res. Commun.* 1975, **67**, 562
11 Alberts, B. M. and Frey, L. *Nature* 1970, **227**, 1313
12 McGhee, J. D. and Von Hippel, P. H. *J. Mol. Biol.* 1974, **89**, 469
13 Kelly, R. C., Jensen, D. E. and Von Hippel, P. H. *J. Biol. Chem.* 1976, **251**, 7240

* Present address: Department of Chemistry, University of Cincinnati, Cincinnati, Ohio 45221, USA.