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THE SYNTHESIS OF POLY(ACRYLIC ACID HYDRAZIDE) AND POLY(METHYLACRYLIC ACID HYDRAZIDE) AND THEIR REACTION PRODUCTS WITH RIBONUCLEOSIDE DIALDEHYDES

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Abstract—Atactic and syndiotactic poly(acrylic acid hydrazide) and atactic, syndiotactic and isotactic poly(methylacrylic acid hydrazide) have been reacted with the di-aldehydes derived from adenosine, guanosine, inosine, cytidine and uridine to give polymers each containing a single type of base residue. Not all of the hydrazide residues of the polymeric hydrazides reacted; guanosine dialdehyde gave the most reaction and inosine dialdehyde the least. Isotactic poly(methylacrylic acid hydrazide) was much less reactive than the other polymeric hydrazides. The adenine-containing and the cytosine-containing polymers with atactic backbones had a low solubility in water whereas those with syndiotactic backbones had a relatively high solubility. For a given polymeric hydrazide backbone the adenine-containing polymers were always the least soluble in water. Most of the ribonucleoside dialdehyde-containing polymers, with the exception of those containing uridine dialdehyde, had only a low solubility in salt solution (0.3M sodium chloride, 0.03M trisodium citrate). No evidence could be obtained for any interaction of these polymers with polynucleotides.

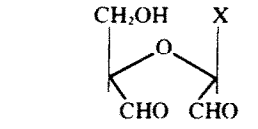
We have previously reported the synthesis of ribonucleoside di-aldehyde derivatives of poly(acrylic acid hydrazide), and have shown that in the case of purine ribonucleoside di-aldehyde derivatives there was interaction in solution with polynucleotides.[1,2] The conditions under which the di-aldehydes react with the polyacrylic acid hydrazide are such that the reaction could be carried out in the presence of a polynucleotide. It appeared, therefore, that it might be possible to obtain conditions such that an added polynucleotide might function as a template and influence the reaction of the di-aldehydes with the polymeric hydrazide and thus confer some specifity on the reaction. The conditions used in the previous work[1,2] were not suitable for this purpose, however, because they led to polymers which contained only low amounts of purine residues (1 purine residue: 10 acrylic acid hydrazide residues) and even fewer pyrimidine residues. The present work was undertaken to obtain polymers with a higher degree of substitution with ribonucleoside di-aldehyde residues. For this purpose, atactic and syndiotactic poly(acrylic acid hydrazides) and atactic, syndiotactic and isotactic poly(methylacrylic acid hydrazides) have been synthesised. These polymeric hydrazides have been condensed with the di-aldehydes obtained by the periodate oxidation of adenosine, guanosine, inosine, cytidine and uridine[3] to give polymers containing purine or pyrimidine side chains.

RESULTS AND DISCUSSION

Ethyl acrylate and methyl methylacrylate were each polymerised by the use of a free radical initiator to give atactic polymers. Syndiotactic poly(ethyl acrylate) and poly(methyl methylacrylate) were made by anionic polymerisation and a sample of isotactic poly(methyl methylacrylate) was a gift from Dr J. N. Hay. For the synthesis of hydrazides of carboxylic acids the method of choice is the reaction of the ester with hydrazine hydrate. Kern *et al.*[4] were the first to obtain polyacrylic acid hydrazide by this method. They treated the polyacrylic ester with hydrazine hydrate; previously they had been unable to isolate the monomer, acrylic acid hydrazide because it cyclised to form a pyrazolidone.[5] In the present work the polymeric hydrazides were obtained by the prolonged treatment of the polymeric esters with hydrazine hydrate. Elemental analysis showed that with the five polymeric esters used, there was incomplete replacement of ester groups by hydrazine residues. It varied from 63% in the case of syndiotactic poly(acrylic acid hydrazide) to 88% in the case of atactic poly(methylacrylic acid hydrazide). There was probably little change in molecular weight of the polymers upon treatment of the esters with hydrazine hydrate so that the molecular weights of the polymeric hydrazides were similar to those given in the experimental section for the polymeric esters. The polymeric hydrazides were usually stored and used in aqueous solution, because it was found that atactic poly(acrylic acid hydrazide), the sample prepared as described in the experimental section and numerous other samples prepared by slightly different procedures, became insoluble in aqueous solutions after freeze-drying. The other polymeric hydrazides did not show this behaviour.

The polymeric hydrazides reacted readily with ribonucleoside di-aldehydes in aqueous solution. The ribonucleoside di-aldehydes react as if they have structure **1**, but there is evidence that in aqueous solution they exist as hydrated forms.[3,6] From evidence obtained from work on the reaction of other hydrazides with ribonucleoside di-aldehydes it can be concluded that the products of the reaction of the polymeric hydrazides with the ribonucleoside di-aldehydes have the structure **2**.

The results given in Table 1 show that although the amount of di-aldehyde added to the polymeric hydrazides was equivalent to the amount of hydrazide residues the reactions did not go to completion. The maximum amount of reaction (71%) was obtained by the reaction of guanosine di-aldehyde with syndiotactic poly(methylacrylic acid hydrazide). Certain general features of the



X = purine or pyrimidine residue

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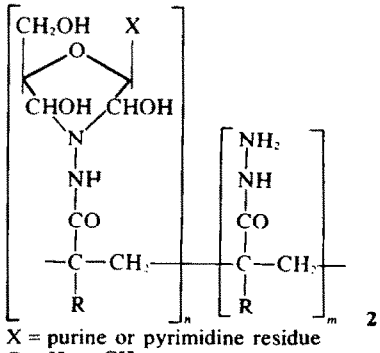

X = purine or pyrimidine residue
R = H or CH_3

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Table 1. Reaction of ribonucleoside dialdehydes with polymeric hydrazides

Polymeric hydrazide	Dialdehyde 1 derived from:	Hydrazide residues reacted (%)	Acrylic residues substituted with 1 (%)	Solubility of product (μmole of 1/ml) In H_2O	In 2 × SCC*
Atactic poly (acrylic acid hydrazide)	Adenosine	54	39	0.032	0.025
	Guanosine	70	51	0.80	0.053
	Inosine	53	38	0.78	0.029
	Cytidine	60	43	0.056	0.045
	Uridine	62	45	0.77	0.097
Syndiotactic poly (acrylic acid hydrazide)	Adenosine	47	30	0.42	0.057
	Guanosine	54	34	0.62	0.16
	Inosine	38	24	⩾0.58	0.56
	Cytidine	43	27	⩾0.53	0.48
	Uridine	50	32	0.71	⩾0.71
Atactic poly(methyl-acrylic acid hydrazide)	Adenosine	45	39	0.041	0.030
	Guanosine	64	56	0.75	0.059
	Inosine	50	44	⩾0.77	0.12
	Cytidine	45	39	0.063	0.053
	Uridine	49	43	0.74	0.70
Syndiotactic poly(methyl-acrylic acid hydrazide)	Adenosine	51	37	0.57	0.042
	Guanosine	71	51	0.78	0.097
	Inosine	50	36	⩾0.76	0.47
	Cytidine	51	37	0.64	⩾0.64
	Uridine	51	37	0.76	0.74
Isotactic poly(methyl-acrylic acid hydrazide)	Adenosine	18	14	0.021	0.020
	Guanosine	28	21	0.13	0.050
	Inosine	12	9	0.030	0.023
	Cytidine	18	14	0.065	0.062
	Uridine	15	11	0.065	0.071

*0.3 M sodium chloride, 0.03 M tri-sodium citrate.

reaction were apparent. Guanosine di-aldehyde reacted to a greater extent with all of the polymeric hydrazides than did the other di-aldehydes and with one exception inosine di-aldehyde reacted to the least extent. Isotactic poly(methylacrylic acid hydrazide) was much less reactive than the other polymers. Examination of molecular models indicated that there is considerable steric hindrance to the formation of polymers which are highly substituted with ribonucleoside di-aldehydes and that this steric hindrance is particularly great in the case of the isotactic polymers. Taking into account the fact that the polymeric hydrazides did not contain 100% hydrazide residues it was calculated that the maximum number of base residues per acrylate residue obtained was 56% [in the case of the guanosine di-aldehyde derivatives of syndiotactic poly(methylacrylic acid hydrazide)]; that in most cases the proportion of base residues present was in the order of 30–40% and that with the derivatives of isotactic poly(methylacrylic acid hydrazide) the proportion was only 9–21%.

Certain conclusions could also be drawn with regard to the solubility of the polymers of structure 2. The adenine-containing polymers with atactic and isotactic backbones had a low solubility in water whereas those with syndiotactic backbones had a relatively high solubility. The same was true in the case of the cytosine-containing polymers. For a given polymeric hydrazide backbone, the adenine-containing polymers were always less soluble in water than the other polymers. Most of the polymers, with the exception of those containing uracil residues had only a low solubility in salt solution (0.3 M sodium chloride, 0.03 M trisodium citrate; 2 × SSC). The presence of the methyl group in the methylacrylate polymers appeared to have little influence on the solubility. Work, not described in detail here, on a number of derivatives of atactic poly (acrylic acid hydrazide) of different molecular weights indicated that molecular weight had little effect on the solubility of the polymers.

Attempts to detect interaction between the polymers of structure 2 and polynucleotides or denatured DNA gave either negative or inconclusive results. This is

rather surprising in view of the interactions observed in previous work.[1,2] The difference in this case was that the polymers obtained were more highly substituted than those previously used so this might impose steric constraints which prevented hybridisation with polynucleotides. The exception is the case of the polymers with an isotactic backbone. Here the degree of substitution was similar to that of the polymers used previously but the isotactic backbone may have imposed more steric constraints than the atactic backbones of the polymers used in the previous work.

From these results it appears, therefore, that the synthesis of polymeric hydrazide-ribonucleoside di-aldehydes under conditions where polynucleotides could act as a template, is unlikely to be successful.

EXPERIMENTAL

General methods. Ultraviolet absorption spectra were measured on a Unicam SP1800 spectrophotometer or a Gilford 2000 multiple absorbance recorder. Gel permeation chromatography was carried out at the Polymer Supply and Characterisation Centre of the Rubber and Plastics Research Association of Great Britain, Shrewsbury, England. From the results obtained $\bar{M}w$ and $\bar{M}n$ were derived by standard procedures. NMR spectra were recorded on either a Perkin Elmer R14 (100 MHz) or a Varian XL-100 spectrometer. ORD spectra were recorded on a F.I.C.A. Spectropol 1 spectropolarimeter.

Atactic poly(ethyl acrylate). To redistilled ethyl acrylate (4 ml) there was added recrystallised azo-bis-isobutyronitrile (5 mg). The reaction mixture was exhaustively de-aerated on a high vacuum line and the solution maintained at 40° for 48 h. The resulting solid was dissolved with difficulty in a large volume of ethanol by vigourous stirring and the viscous solution poured into a large excess of heptane. The resulting precipitate was collected and dried *in vacuo*. (PA-1, 3.5 g) $\bar{M}w$, 5.3×10^6; $\bar{M}n$, 1.7×10^6.

Syndiotactic poly(ethyl acrylate). Dry tetrahydrofuran (100 ml) and redistilled ethyl acrylate (10 ml) were distilled onto a solution of butyl lithium in hexane (2 ml of a 2.2 M solution) frozen in liquid nitrogen. The polymerisation mixture was allowed to warm slowly to −80° with stirring and then kept at this temperature for 12 h. The temperature was raised to 20° and the solution kept for a further 12 h and then methanol (10 ml) added and the resulting viscous solution poured with stirring into heptane (300 ml). The resulting suspension was centrifuged at 5000 rpm for 10 min and the sediment dried *in vacuo* at 90° to give the required polymer (PA-2, 0.8 g). This gave solutions in tetrahydrofuran which at the required concentrations were too viscous to carry out gel permeation chromatographic analysis or NMR spectroscopy. It was assumed, however, that the polymer was of very high molecular weight and that because of its method of preparation that it was mainly syndiotactic.

Atactic poly(methyl methylacrylate). Methyl methylacrylate (4 ml) was distilled onto a solution of recrystallised azo-bis-isobutyronitrile (5 mg) in octane (1 ml) in a high vacuum system. The solution was deaerated and then maintained at 40° for 48 h. After this time a hard white solid was obtained below a layer of octane. The octane was decanted off and the residual solid dissolved in chloroform (500 ml) by shaking for 24 h. The solution was then poured with stirring into a large excess of heptane. The resulting white solid was centrifuged off, dried *in vacuo* and ground into a white powder (PMA-1, 3.8 g) $\bar{M}w$, 9.5×10^6, $\bar{M}n$, 3.4×10^6.

Syndiotactic poly(methyl methylacrylate). Dry tetrahydrofuran (100 ml) and dry methyl methylacrylate (10 ml) were distilled in a vacuum line onto a solution of butyl lithium in hexane (2.5 ml of a 2.2 M solution) which had been deaerated and cooled in liquid nitrogen. The mixture was kept at −80° with stirring for 12 h, allowed to warm to room temperature and the reaction quenched by the addition of methanol. The solution was then poured into a large volume of heptane to give a white precipitate of the required polymer. This was centrifuged off and dried at 90° *in vacuo* (PMA-2, 8.9 g) $\bar{M}w$, 3.3×10^4; $\bar{M}n$, 1.0×10^4. The NMR spectrum of this polymer in $CDCl_3$ corresponded precisely to that of Bovey and Tiers[8] for a syndiotactic polymer.

Isotactic poly(methyl methylacrylate). This sample was supplied by Dr J. N. Hay of this Department. It had been obtained by the anionic polymerisation of methyl methylacrylate with phenylmagnesium bromide in toluene. The sample (PMA-3) was transparent and crystalline $\bar{M}w$, 10^5.

Atactic poly(acrylic acid hydrazide). Atactic poly(ethyl acrylate)(PA-1, 0.5 g) was dissolved in ethanol (150 ml) and hydrazine hydrate (150 ml) added. The mixture was boiled under reflux until it was no longer turbid and then the resulting clear solution was distilled until 80 ml of the solvent had been removed. Hydrazine hydrate (80 ml) was then added and the solution boiled under reflux for 48 h. The solution was then distilled to remove 70 ml of solvent, hydrazine hydrate (100 ml) added and the solution boiled under reflux for 48 h. Water was then added and the solution dialysed against running water for 3 days, against 0.1 M EDTA for 1 day and then against several changes of distilled water for several days. The material inside the dialysis bag was then filtered through muslin to remove gel-like material and then through filter paper. A sample of the filtrate (50 ml) was freeze-dried, but the remainder of the product was stored in solution. The total yield of atactic poly(acrylic acid hydrazide) (PAAH-1) was 0.4 g. The freeze-dried material would not redissolve in water. (Found: N, 21.8; H_2O, 7.8; ash < 1%). Hence 73% of the ester residues of the polymer have been replaced by hydrazide residues.

Syndiotactic poly(acrylic acid hydrazide). Syndiotactic poly(ethyl acrylate) (PA-2, 0.5 g) was dissolved in hydrazine hydrate (250 ml) with stirring. The solution was boiled under reflux for 24 h, cooled, water (200 ml) added and the solution dialysed as described above. The material remaining in the dialysis bag was completely soluble. A sample of this solution was freeze-dried and this readily redissolved in water. The total yield of syndiotactic poly(acrylic acid hydrazide) (PAAH-2) was 0.36 g. (Found: N, 18.2; H_2O, 11.2%). Hence 63% of the ester residues of the polymer were replaced by hydrazide residues.

Atactic poly(methylacrylic acid hydrazide). Atactic poly(methyl methylacrylate) (PMA-1, 3.8 g) was dissolved with stirring in hydrazine hydrate (500 ml) and the solution boiled under reflux for 7 days. To the cooled solution, water (500 ml) was added and the solution dialysed as described above. The solution inside the dialysis bag was filtered to remove a small amount of insoluble material. A sample of atactic poly(methylacrylic acid hydrazide) (PMAAH-1) was obtained by freeze-drying; this could be redissolved in water. (Found: N, 21.0; H_2O, 11.0%). Hence 88% of the ester residues of the polymer were replaced by hydrazide residues.

Syndiotactic poly(methylacrylic acid hydrazide). Syndiotactic poly(methyl methylacrylate) (PMA-2, 1 g) was dissolved in hydrazine hydrate (250 ml) and the solution boiled under reflux for 72 h. The reaction mixture was then treated in the same manner as for PAAH-2 to give syndiotactic poly(methylacrylic acid hydrazide (PMAAH-2, 0.9 g). A sample of the material was obtained by freeze-drying. This was readily soluble in water. (Found: N, 16.8; H_2O, 16.3%). Hence 72% of the ester residues of the polymer had been replaced by hydrazide residues.

Isotactic poly(methylacrylic acid hydrazide). Isotactic poly(methyl methylacrylate) (PMA-3, 3.5 g) was dissolved in hydrazine hydrate (500 ml) and the solution boiled under reflux for 48 h. The isotactic poly(methylacrylic acid hydrazide) (PMAAH-3, 2.6 g) was isolated in the usual way. A freeze-dried sample was soluble in water. (Found: N, 19.2; H_2O, 8.7%). Hence 76% of the ester residues of the polymer were replaced by hydrazide residues.

Reaction of the polymeric hydrazides with ribonucleoside di-aldehydes. The ribonucleoside di-aldehydes were prepared as described previously.[3] A solution of a particular ribonucleoside di-aldehyde in water (3.0 μmole/ml) was mixed with an equal volume of an aqueous solution of a polymeric hydrazide (containing 3.0 μmole/ml of hydrazide residues) and the solution allowed to stand at 20° for 24 h. Examination of the solution at this stage by TLC in butan-1-ol-ethanol-water (4:1:5, organic

phase) showed that in every case there was present a UV-absorbing component which remained on the base line (the required polymeric product) and a spot corresponding to unreacted ribonucleoside di-aldehyde. The latter did not decrease after extended reaction times; in no case did all of the hydrazide residues react. The solution was then dialysed exhaustively against distilled water to give the required polymer of structure **2** inside the dialysis bag.

The procedure described above was carried out using the di-aldehydes derived from adenosine, guanosine, inosine, uridine and cytidine. Each of these ribonucleoside di-aldehydes was reacted with the five polymeric hydrazides whose preparation is described above. The extent of the reaction was determined by measuring the UV absorption of the liquid in the dialysis bag (a correction was applied to take account of a small UV absorbance of the polymeric hydrazide). In most this was not a true solution but a fine suspension, but it was found that this procedure still gave satisfactory results. This was checked in a number of cases by hydrolysing the material to free purines or pyrimidines and determining these by standard procedures. The amount of water-soluble polymer formed was determined by measuring the UV absorption of the supernatant liquid obtained after centrifuging at 100000 g until a clear solution was obtained. These aqueous solutions were then adjusted to 0.3 M with respect to sodium chloride and 0.03 M with respect to sodium citrate and the resulting suspensions centrifuged until clear. The UV absorption of the supernatant liquid gave the solubility of the polymers in this salt solution (2 × SSC). The results are shown in Table 1.

Interaction with polynucleotides of the ribonucleoside di-aldehyde derivatives of the polymeric hydrazides. Attempts were made to detect the interaction of the polymers of structure **2** with homopolyribonucleotides by measuring the hypochromic effect obtained upon mixing the appropriate polymers.[2] Possible interaction with denatured DNA and between complementary pairs of the synthetic polymers were also investigated by the same procedure. In no case, where the polymers remained in solution, was a hypochomic effect observed. In a few cases it appeared that the synthetic polymer caused precipitation of the polynucleotide. Attempts were also made to detect interaction by measuring the ORD curves of mixtures of the synthetic polymers with polyribonucleotides. These curves were simply additive of those of the two constituents, however, so there was no evidence of interaction.

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