Carbohydrate Polymers 5 (1985) 473–478

Research Note

Radiation Effects on the Biological Activity and Molecular Weight Parameters of Heparin

Ionizing radiations are commonly used in grafting techniques, whereby the radiation-induced and long-lived radicals generated initiate chain reactions when mixed with suitable monomeric components. Heparin can be grafted (Shimada *et al.*, 1985) and subsequently used in biomedical devices or clinical implants and it follows that the effect of the initial dose of radiation on the 'integrity' of heparin is a major consideration. It is also possible that ionizing radiations, in combination with other sterilizing techniques, could be used during the pharmaceutical production of heparin. All these emerging techniques and ideas require a direct correlation between biological activity and physical changes, which is the subject of this investigation.

Heparin samples of three different anticoagulant activities were used in this study: high activity (HA) 174·6 USP units mg^{-1}, H-3125 (Sigma Chemical Company Ltd, USA), medium activity (MA) 108·1 BP units mg^{-1}, fraction SH1 R275 (Glaxo, UK) and low activity (LA) 74·3 USP units mg^{-1} (Abbott Laboratories, USA). All heparin samples were dried *in vacuo* for 48 h before being sealed in tubes under vacuum and irradiated with ^{60}Co gamma radiation at a dose rate of 10 kGy h^{-1} to four doses: 5, 10, 15 and 20 kGy total dose and an additional 30, 50, 80 and 100 kGy total dose for the high activity sample.

The anticoagulant activities of the heparin samples were determined using the British Pharmacopoeia (BP) (1980) 1973 method and the 1983 Manchester (National (UK) Reference Laboratory for Anticoagulant Reagents and Control) standardised activated partial thromboplastin time (APTT) (Thomson, 1980) with the Third International Reference Preparation of Heparin (WHO) being used as standard in both assays. All reagents were of Analar grade. For the APTT assay, 0·3 units ml^{-1}

Carbohydrate Polymers 0144-8617/85/$03.30 – © Elsevier Applied Science Publishers Ltd, England, 1985. Printed in Great Britain

solutions of all the heparins were prepared with the activity based on the BP value of the unirradiated heparin sample. Changes in activity were thereby presented as % change compared to the unirradiated samples.

Molecular weight estimations of heparin were carried out by high pressure liquid chromatography (HPLC, Perkin–Elmer, USA) Harenberg & De Vries, 1983) using a column (Ultrapac TSK G3000 SW, 600 × 7·5 mm i.d., particle size 10 ± 2 μm; LKB No. 2135-360), a detector (Differential Refractometer R401, Waters) and a two-channel recorder. A pre-column (Ultrapac TSK GSWP, 75 × 7·5 mm i.d., 10 μm LKB) was connected between the pump and the main column. The mobile phase was 0·1 M phosphate buffer (pH 7·0; filtered (0·45 μm) and degassed before use). The column was calibrated using heparins of defined molecular weights (provided by Dr Grant Barlow, Abbott Laboratories, USA). The degassed and filtered 0·1 M phosphate buffer was used to prepare a 3% solution of heparin which was passed through a 0·45 μm filter before 60 μl were injected onto the column. The molecular weight parameters were calculated using a Perkin–Elmer Basic program (GPC 2L).

Figure 1 shows the chromatograms of the three heparin samples for which the molecular weight parameters were calculated. The samples varied in their biological potency and commercial source. The MA sample had been isolated by an alcohol fractionation process, whereas the LA heparin was a crude extract containing other glycosaminoglycans such as dermatan sulphate and heparan sulphate. The twin peak profile in the chromatogram of LA (Fig. 1) as well as the polydispersity value (Table 1) indicate the presence of non-heparin components.

Irradiation of these samples to a total dose of 20 kGy (Table 1) produced no major change in their biological potency as assessed using the BP method and APTT assay. With the former test less than a 2% decrease in activity was obtained for the three samples. With the APTT assay a slight increase in activity was noted for HA, whilst MA and LA heparins were similar to the trend with the BP method. In contrast to the anticoagulant activity data, all the molecular weight parameters decreased following irradiation, indicating some degradation and glycosidic bond scission. For heparin, the molecular weight distribution varies according to origin, isolation and purification methods, fractionation and treatment with reagents (Jaques *et al.*, 1973; Shen *et al.*, 1978). Size exclusion liquid chromatography has been used in this

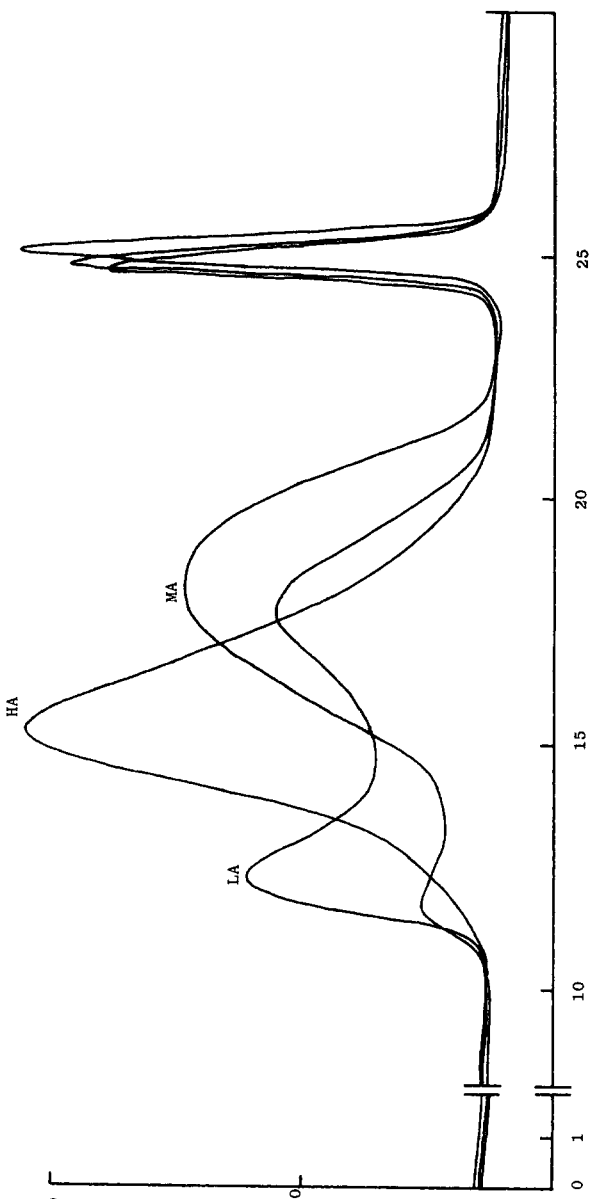

Fig. 1. Chromatogram of heparins: LA, low activity, 74·3 units mg^{-1}; MA medium activity, 108·1 units mg^{-1}; and HA, high activity, 174·6 units mg^{-1}.

TABLE 1

Changes in the Anticoagulant Activities and Molecular Weight Parameters of Heparins with Increasing Doses of Ionizing Radiations (LA, MA and HA represent low, medium and high activity heparin)

Dose (kGy)	*Sample*	*BP*[a] *(units mg^{-1})*	*APTT*[b]	M_W[c]	M_N[d]	M_Z[e]	Q[f]
0	HA	174·63 (176·41–172·86)	100·0% 58·5	12 750	10 980	14 660	1·16
	MA	108·05 (109·91–106·20)	100·0% 65·5	9 570	7 200	13 960	1·33
	LA	74·29 (75·36–73·23)	100·0% 60	14 560	10 260	19 460	1·42
5	HA	174·65 (178·42–170·92)	103·4% 60·5	12 320	10 430	14 330	1·18
	MA	107·88 (109·05–106·73)	100·0% 65·5	9 370	7 100	13 570	1·32
	LA	73·44 (74·59–72·31)	99·2% 59·5	13 950	9 890	18 790	1·41
10	HA	172·86 (173·94–171·79)	104·7% 61·25	12 200	10 290	14 310	1·19
	MA	107·23 (108·26–106·21)	101·7% 66·5	9 220	7 110	13 080	1·30
	LA	73·73 (75·41–72·09)	98·3% 59	13 940	9 850	18 800	1·42
15	HA	174·75 (175·95–173·56)	105·9% 62	11 530	9 550	13 600	1·21
	MA	106·84 (108·66–105·05)	100·8% 66	8 870	6 820	12 650	1·30
	LA	72·93 (73·92–71·96)	99·2% 59·5	14 100	10 020	18 820	1·41
20	HA	174·00 (175·23–172·78)	104·3% 61	11 260	9 240	13 320	1·22
	MA	107·32 (108·21–106·43)	102·3% 67	8 970	6 840	12 930	1·31
	LA	72·82 (73·47–72·18)	99·2% 59·5	13 460	9 540	18 210	1·41

[a] Values in parentheses are fiducial limits.

[b] Each value is the average of two assays within a range ±1 s and clotting times at 0 dose are taken at 100%.

[c] Weight average molecular weight.

[d] Number average molecular weight.

[e] Charge average molecular weight.

[f] Polydispersity.

study to determine the molecular weight of heparin since highly purified heparin oligosaccharides were available as calibration standards. This present study shows that following a dose of 20 kGy of ionizing radiation all three molecular weight parameters decrease (in a range 5–15·8%) (Table 1). This technique shows that radiation caused degradation of heparin, but since the heparin was irradiated in the dry solid state the changes are not so pronounced as to affect the anticoagulant activity.

In order to decrease the biological activity a higher radiation dose was required. Table 2 shows the radiation-induced degradation observed using the BP method and the size exclusion liquid chromatography technique. At doses as high as 100 kGy, heparin still retained 81·3% of its original activity and the molecular weight parameters showed similar changes. In contrast, Chawla & Hayward (1980) detected a 10% decrease in the anticoagulant activity of an aqueous solution of heparin following a dose of 9·2 kGy. Although, as the present work shows, different heparins are more susceptible to radiation damage than others, it is the absence of water from the heparin samples that is mainly responsible for protection against radiation damage. The radiolysis of carbohydrates in aqueous solution occurs mainly by the reaction of OH radicals formed by water radiolysis ($k \sim 1 \times 10^9$ M^{-1} s^{-1}).

TABLE 2

Changes in the BP Anticoagulant Activity and Molecular Weight Parameters for Glaxo Heparin following 100 kGy Total Radiation Dose.

Dose (kGy)	*BP*[a] *(units mg^{-1})*	M_W[b]	M_N[c]	M_Z[d]	Q[e]
0	105·37 (106·83–103·94)	9 250	7 160	13 210	1·29
30	102·17 (103·57–100·78)	8 580	6 590	12 270	1·30
50	97·80 (99·65–95·99)	8 100	6 380	11 100	1·27
80	84·81 (86·06–83·58)	7 810	6 110	10 830	1·28
100	76·25 (77·19–75·31)	7 670	6 060	10 400	1·27

[a] Values in parentheses are fiducial limits.
[b] Weight average molecular weight.
[c] Number average molecular weight.
[d] Charge average molecular weight.
[e] Polydispersity.

Subsequent carbohydrate radicals formed are capable of undergoing further reactions such as elimination and rearrangements to produce secondary carbohydrate radicals (Kochetkov *et al.*, 1979). The yield and nature of these radicals depends on the environment and phase of the carbohydrate molecule. In the present work irradiations were performed *in vacuo* using previously dried heparin, with the result that such oxidative degradative changes are mainly eliminated. Such a result has implications for the pharmaceutical use of radiation-sterilized heparin and heparinized surfaces.

ACKNOWLEDGEMENTS

The authors are grateful to the World Health Organisation for the supply of reference heparin and to the National (UK) Reference Laboratory for Anticoagulant Reagents and Control for cephalin reagent. One of us (HEE) acknowledges the support of Mombusho.

REFERENCES

British Pharmacopoeia (1980). 1973 Method, Appendix XIVD A147, London, HMSO.

Chawla, A. T. & Hayward, C. (1980). *Pharmacology* **20**, 224.

Harenberg, J. & De Vries, J. X. (1983). *J. Chromatogr.* **261**, 287.

Jaques, L. B., Kavanagh, L. W. & Kuo, S. H. (1973). *Thromb Res.* **3**, 295.

Kochetkov, N. K. Kudrjashov, L. I. & Chlenov, M. A. (1979). *Radiation Chemistry of Carbohydrates*, Oxford, Pergamon Press, pp. 141-207.

Shen, L. L., Barlow, G. H. & Holleman, W. H. (1978). *Thromb. Res.* **13**, 671.

Shimada, M., Takigami, S., Nakamura, Y., Edwards, H. E. & Phillips, G. O. (1985). *J. Appl. Polymer Sci.* (submitted).

Thomson, J. M. (1980) Ed. *Blood coagulation and haemostasis*, Edinburgh, Churchill Livingstone.

H. E. Edwards, A. R. Menzies and G. O. Phillips
Research Division,
The North East Wales Institute,
Deeside, Clwyd CH5 4BR, Wales, UK

Y. Nakamura, M. Shimada and S. Takigami
Faculty of Technology,
Gunma University,
Kiryu, Gunma 376, Japan

(Received: 17 January 1985)