Short Communications

SC 2190

The determination of the molecular weight of poly-L-lysine

In our recent investigations on multi-chain polyamino acids[1,2], poly-L-lysine was employed as a multivalent initiator of polymerization. In order to derive the molecular parameters of these materials, in which polylysine forms the "backbone", it was esssential to have an accurate knowledge of the average degree of polymerization of any given sample of this polyamino acid. Although poly-L-lysine hydrobromide (or hydrochloride) has been used quite extensively in chemical and biological research[3,4], it seems that no satisfactory method has been reported by which its molecular weight can be evaluated. The reason for this situation is that polylysine, a basic polyelectrolyte, is soluble only in its ionized form; free polylysine has been prepared and was found to be insoluble in all except acidic ionizing solvents. Direct molecular-weight determinations of highly charged macromolecules by physical means meet with many complications of both theoretical and experimental nature. The present practice is to assume that a polylysine sample has the same degree of polymerization as the polycarbobenzoxylysine preparation from which it was derived. This assumption may often be valid, *i.e.* when decarbobenzoxylation was carried out under mild and well controlled conditions[5] (see also Table I), but may sometimes be a source of serious error[5]. From Table I it can be seen that even under presumably favorable conditions

TABLE I

Batch No.	*Parent polycarbobenzoxylysine*		*Poly-L-lysine hydrobromide**	*Polycarbobenzoxylysine by recarbobenzoxylation***	
	[η] (dl/g)	*DP****	*η_{sp} (H_2O, 1%)*	*[η] (dl/g)*	*DP****
7	2.1	1290	6.2	1.95	1260
5	0.9	680	2.52	0.76	565
8	0.35	317	0.92	0.33	300
2	0.37	325	0.76	0.27	252
3	0.17	175	0.55	0.17	175
6	0.11	126	0.42	0.11	126
4	0.08	96	0.23	0.08	96

* Poly-L-lysine hydrobromide was prepared from the parent poly-ε-carbobenzoxy-L-lysine by the method of BEN-ISHAI AND BERGER[7]. The reaction time was 20 min at room temperature.

** Poly-L-lysine hydrobromide (364 mg), was dissolved in water (6 ml). To the cooled and stirred solution, 4 N NaOH (2.25 ml) and benzylcholoroformate (0.51 ml) were added alternately in 3 portions. The product precipitated out after the first addition of the acid chloride. Stirring was continued for 10 min. The polymer was collected, washed thoroughly with water, ethanol and ether, and dried *in vacuo* over H_2SO_4. Yield 90%. Calcd. for $C_{14}H_{18}N_2O_3$: N, 10.7. Found: N, 10.6; 10.8 (Kjeldahl). The polymer contained no basic groups as determined by anhydrous titration in dimethylformamide solution with $HClO_4$ in dioxane, using thymol blue as indicator.

*** Degree of polymerization as estimated from viscosity measurements in dimethylformamide[6].

Biochim. Biophys. Acta, 69 (1963) 397–399

degradation may occur for unknown reasons. Moreover, with polymers of high degrees of polymerization, even a low percentage of bonds split may have a marked effect on the average molecular weight of the product. It is, therefore, important to have a way of checking the molecular weight of a polylysine preparation directly be it for reasons given above or because data on the parent polycarbobenzoxylysine preparation are not available. In this communication two alternative procedures are described which may serve this purpose.

In the first method, which can be applied to any poly-L-lysine preparation, the polylysine sample is converted into polycarbobenzoxylysine* (see footnote in Table I). The reaction is practically instantaneous and the product is isolated in high yield, being analytically pure after washing and drying. The intrinsic viscosity $[\eta]$ of this polymer is then determined in dimethylformamide solution and its degree of polymerization, *DP*, calculated from the equation[6] $DP = (10^7[\eta]/2.24)^{-1.26}/262$. Because of the extremely mild conditions of the recarbobenzoxylation and the short reaction time involved, it can be assumed** that the degree of polymerization thus obtained is identical with that of the polylysine sample used.

The second method is based on viscosity measurements of polylysine hydrobromide in salt-free aqueous solution. Employing the values of the degrees of polymerization of a number of polylysine hydrobromide samples determined by the first method, the following relationship was established:

$$\log DP = 0.79 \log \eta_{\mathrm{sp}(c=1\%)} + 2.46$$

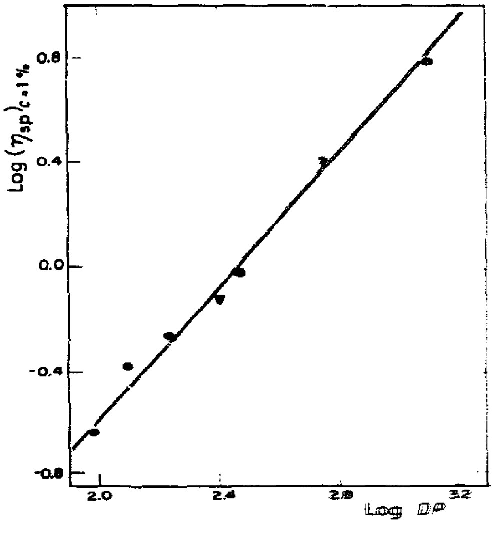

Fig. 1. The log of the degree of polymerization (log *DP*) of poly-L-lysine·HBr against log of the specific viscosity (log η_{sp}) at $c = 1\%$ in water.

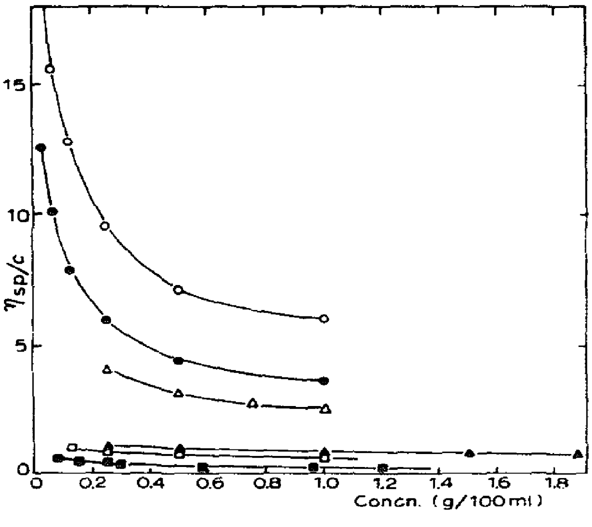

Fig. 2. The reduced viscosity (η_{sp}/c) of some poly-L-lysine·HBr samples in water, plotted against the concentration (c).

---

* A similar approach, *i.e.* conversion of a polyelectrolyte into an uncharged derivative, has been used by KATCHALSKY AND EISENBERG[9].

** An indirect confirmation of this assumption can be obtained from Fig. 1. All the batches marked by circles (3, 4, 6, 7, 8 in Table I) show no degradation. Batches 2 and 5, marked by triangles were degraded. The fact that they lie on the same straight line as the undegraded samples shows that degradation occurred during the HBr treatment and not during recarbobenzoxylation.

The linearity of this function in the range of degree of polymerization $DP = 100$–1000 can be seen in Fig. 1. The conditions of the viscosity measurements were chosen because of the following considerations. In Fig. 2 the concentration dependence of a number of polylysine·HBr samples in salt-free aqueous solution is given. It has been demonstrated that extrapolation of curves of this kind to $c = 0$ in order to obtain intrinsic viscosities is not feasible[8]. The concentration of 1% was chosen so as to avoid working in the steep region of the curve, and in the absence of added salt viscosity values of reasonable magnitude are obtained even for polymers of rather low molecular weight ($DP = 100$).

The most serious drawback of the second method lies in the following peculiar behavior of poly-L-lysine·HBr, which is as yet not understood. The polymer when kept at —15° shows complete stability of its viscosity properties. However, at room temperature certain changes take place which result in viscosity increases. Thus the value of $\eta_{\mathrm{sp}\,(c\,=\,1\%)} = 2.5$ in water of a freshly prepared sample rose to 4.6 after keeping it in a desiccator at room temperature for four months. This change is not due to an increase in molecular weight of the sample, as can be seen from the fact that the polycarbobenzoxylysine preparations made from the fresh and from the aged material had the same viscosity. It is therefore clear that if a polylysine sample has been kept under unfavorable or unknown conditions for a considerable length of time, the first procedure (recarbobenzoxylation) for the determination of its degree of polymerization must be employed.

This work was supported by a research grant (FG-Is-104-60) from the U.S. Department of Agriculture.

Department of Biophysics, The Weizmann Institute of Science,
Rehovoth (Israel)

ARIEH YARON
ARIEH BERGER

[1] M. SELA, E. KATCHALSKI AND M. GEHATIA, *J. Am. Chem. Soc.*, 78 (1956) 746.
[2] A. BERGER AND A. YARON, in M. A. STAHMANN, *Polyamino Acids, Polypeptides and Proteins*, The University of Wisconsin Press, Madison, 1962, p. 13.
[3] E. KATCHALSKI AND M. SELA, *Advan. Protein Chem.*, 13 (1958) 243.
[4] M. SELA AND E. KATCHALSKI, *Advan. Protein Chem.*, 14 (1959) 391.
[5] G. D. FASMAN AND E. R. BLOUT, *J. Am. Chem. Soc.*, 83 (1961) 709.
[6] E. DANIEL AND E. KATCHALSKI, in M. A. STAHMANN, *Polyamino Acids, Polypeptides and Proteins*, The University of Wisconsin Press, Madison, 1962, p. 183.
[7] D. BEN-ISHAI AND A. BERGER, *J. Org. Chem.*, 17 (1952) 1564.
[8] H. EISENBERG AND J. POUYET, *J. Polymer Sci.*, 13 (1954) 85.
[9] A. KATCHALSKY AND H. EISENBERG, *J. Polymer Sci.*, 6 (1951) 145.

Received June 13th, 1962

Biochim. Biophys. Acta, 69 (1963) 397–399

SC 2193

Mitochondrial formation and hydrolysis of adenosine triphosphate in the presence of some rare-earth ions

The intravenous injection of relatively small amounts of soluble salts of lanthanum, cerium, praseodymium, neodymium, and samarium causes in some species of experimental animals, after a lag period of 2 or 3 days, an extensive accumulation of lipids in the liver[1–5]. It is remarkable that these lanthanide ions produce a large increase in triglycerides and a smaller rise of phospholipids of the mitochondria, in

Biochim. Biophys. Acta, 69 (1963) 399–402