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LETTER TO THE EDITORS-IN-CHIEF

POLYLYSINE AGGREGATION OF HUMAN BLOOD PLATELETS

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The importance of looking for molecular factors that might be critical in aggregation phenomenae of blood platelets cannot be denied. Metcalf and Lyman (1) have implied that the conformational state of different samples of poly-L-lysine (PLL), in normal saline, is different, and that this difference results in different interaction of the PLL samples in their ability to aggregate platelets. We do not agree with these conclusions.

In the first place, all samples of PLL available to us gave identical circular dichroism (CD) spectra to each other when dissolved in the same media. We have tested the L polymer as supplied by New England Nuclear, Cambridge, Mass., lot L-100 (mol. wt. 80,000), lot L-80 (mol. wt. 120,000), lot 1-77 (mol. wt. 100,000), as well as two obtained from Sigma Chem. Co. St. Louis, Mo. lot 19-B-5280 (mol. wt. 2800), and lot 34 C-5300 (mol. wt. 85,000). All PLL samples dissolved in water gave a small + CD band at 218 nm and a 10 x larger - band in the 195 nm region (2). The effects of NaCl on the CD spectra of PLL recently has been examined extensively in model studies directed at the effects of salts on the conformation of the polypeptide backbone (2). In 0.15 M NaCl, the repulsion between positively charged side-chains was reduced by shielding, allowing some collapse from an extended state. Higher concentrations of NaCl introduced some helicity. In no case, however, were any of the CD curves of PLL in NaCl similar to those displayed by Metcalf and Lyman (1).

In addition, we observed identical platelet aggregation curves with all the samples of PLL, as well as with poly DL lysine (Sigma Chemical Co., lot 123 C-5280, mol. wt. 64,000), when the same experimental conditions were employed. Using human platelet rich plasma, we obtained a two phase transition similar to that recorded by Massini and Lüscher (3). However, platelets washed 3 x and suspended in a buffer according to the method of Metcalf and Lyman (1) produced a simple immediate increase in light transmission and aggregation curves which were similar to those reported by Jenkins, et al. (4), who used only the D, L polymer. Massini and Lüscher (3) noted that the

addition of only 2% plasma to washed platelets could increase the transmission from 15% to 37% while at the same time increasing the serotonin release from 5% to 67%. Thus, the degree of removal of absorbed plasma components by different methods of washing, could be the critical factor in aggregation obtained by different research groups (1,3,4,5), and not the conformation of the polylysine. Critical to this conclusion is the independence of platelet aggregation from effects due to the introduction of D groups in the L polymer which prevent the same ordered conformations obtained when only the L groups are present.

Recently it has been stated that PLL at high concentrations loses its effectiveness to aggregate platelets (6), since light transmission changes were less when a high concentration of polymer was used to produce aggregation. We find, however, that traces of formalin, leaching out of fixed platelets caused precipitation of PLL. Thus the measured light transmission is a balance between increased light due to platelet aggregation and decreased light due to additional particulate PLL particles. All experimental aggregation curves may be subject to misinterpretation as a result of such counter effects.

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