AGGREGATION OF PLATELETS BY FISH COLLAGEN: EFFECT OF SIDE CHAIN ACETYLATION

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PLATELETS adhere to perivascular and sub-endothelial tissues led to the recognition that collagen, a major component of perivascular connective tissue, is involved in platelet adhesion, activation and hemostasis[1-5]. It is reported that collagen polymer having a particle size of about three molecular lengths (7000 to 18000 Å) is the minimum length required to induce platelet aggregation[6]. The platelet reactive sites in both α_1 (1) and α_2 (1) chains of type I collagen have been established[7]. Type I collagen appeared to contain many platelet aggregatory sites of different reactivity distributed along the length of the molecule. Each site was thought to involve two or more basic residues comprising of lysine located in the adjacent chains and the specific orientation of one to the other. The triple helical structure of the collagen molecule seems to be crucial for platelet aggregating activity.

If this is true, experimental modification of collagen should throw more light on the physical and chemical properties necessary for the interaction of collagen with platelets. The free amino groups of lysine are reported to be critical for the platelet-aggregating activity[3,8-10]. Wilner[10] suggested that collagen must possess free amino groups since blockage of these groups by deamination, N-acetylation or treatment with dinitroflurobenzene resulted in over 90% reduction of platelet-aggregating activity. However, Wang *et al*[11] reported that when acetylated native type fibrillar collagen is added to platelet-rich plasma, platelet aggregation occurred to the same degree as that was seen with an equal amount of unmodified fibrillar collagen. In this communication, we present evidence that blocking of epsilon amino groups of lysine by acetylation reduces platelet-aggregating activity of collagen from fish skin (lower vertebrate).

Venous blood was drawn from normal subjects using disposable needles and polypropylene syringes. The blood was anticoagulated with 0.1 volume 4% trisodium citrate and processed in polystyrene tubes.

Platelet-rich plasma was prepared by centrifuging at room temperature[12,13] for 20 min at 500 *g*. The supernatant plasma was transferred to a polystyrene tube using siliconized pipette.

Platelet-poor plasma was prepared by centrifuging the platelet depleted plasma at 6000 *g* for 2 h at 4°C.

Acetylation of collagen was performed by reacting collagen[14] (5 g) suspended in ethyl acetate (50 ml) with excess of acetic anhydride (30 ml) in the presence of 3 ml of 90% formic acid, which acted as a catalyst. This reaction mixture was allowed to stand for 7 days after which, the collagen was washed and dehydrated in acetone[15]. The acetylated collagen was then neutralized in 0.02 M Na_2HPO_4 and freeze-dried.

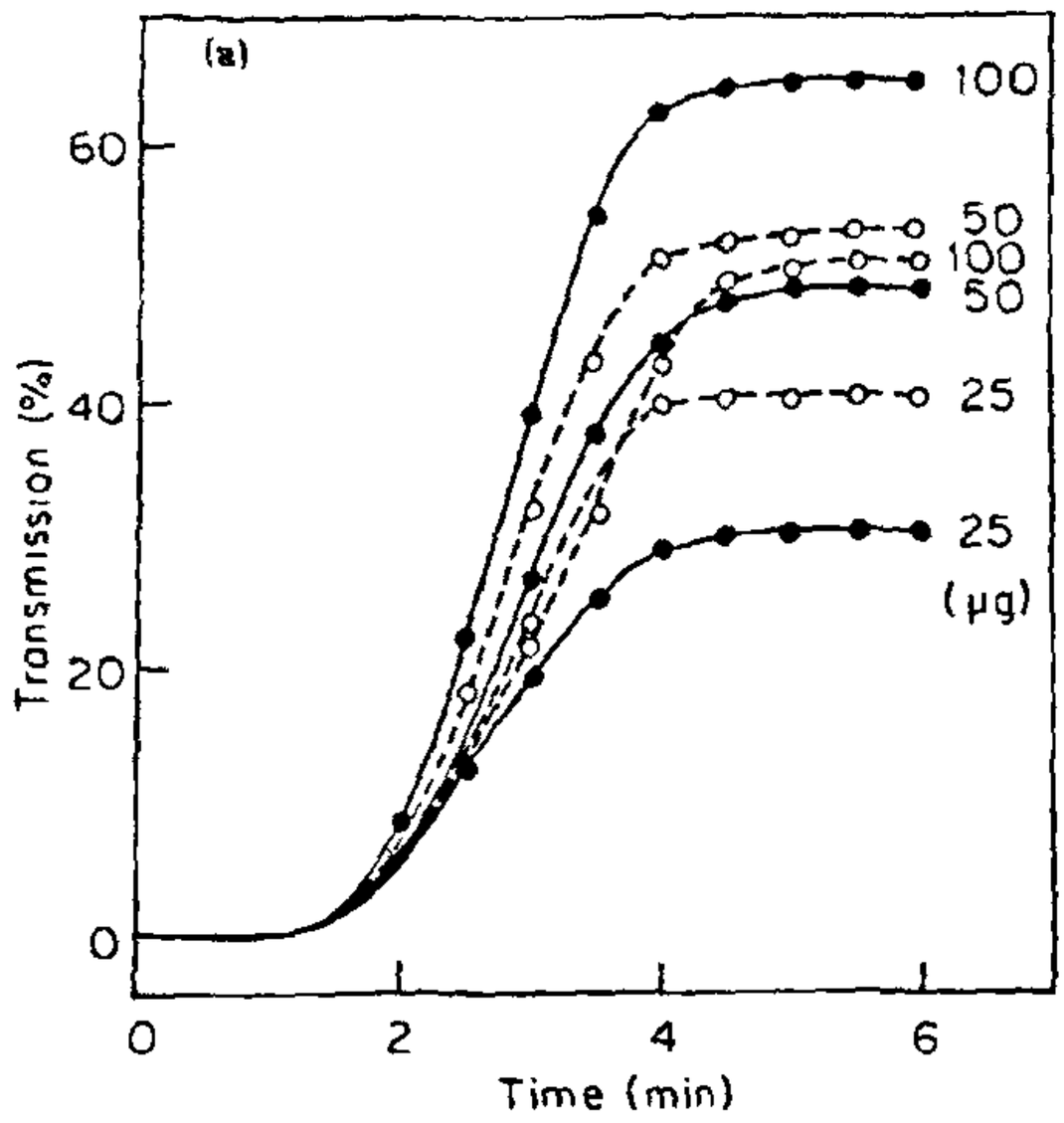
Platelet aggregation was measured by a turbidimetric method[16]. Platelet-rich plasma (1.2 ml) was mixed with 0.1 ml of saline in a siliconized cuvette and stirred for 1 min, followed by the addition of 0.1 ml of collagen solution at 37°C (collagen concentration was maintained between 25 μg and 100 μg/ml). Aggregation was recorded in a Shimadzu recorder at every 30 s interval as the change in the transmittance.

The blank consisted of 0.3 ml of saline and 1.2 ml of platelet-poor plasma from the same blood sample.

The platelet aggregation curves were analysed by dividing the curve into lag and aggregation phases. The lag phase extends from the point of collagen addition to the point where the curve is elevated from the baseline by one transmission unit. The slope of the aggregation phase is defined as the rate of change in light transmission units between the baseline and asymphotic part of the curve.

The results of the study are given in figure 1a, b. Each figure shows results of platelet aggregation as studied with two concentrations of platelet: 400,000/mm^3 and 200,000/mm^3. The results show that platelet aggregation in acetylated collagen is reduced (between 50 and 20%) at all the concentrations of collagen (25 μg to 100 μg) tested compared to the control. It is also seen that there is prolongation in the completion of the lag phase in acetylated collagen thereby showing that acetylation restricts platelet aggregation. In our study, we could not observe complete loss of platelet aggregation. This can be explained by the fact that the requirement of minimum length of collagen molecule (7000-18000 Å) is still available in acetylated collagen to induce platelet aggregation. Thus, the decreased platelet aggregation can be ascribed to acetylation.

Fish collagen was found to have the highest platelet-aggregating activity when compared to invertebrate collagen[12] and the higher vertebrate



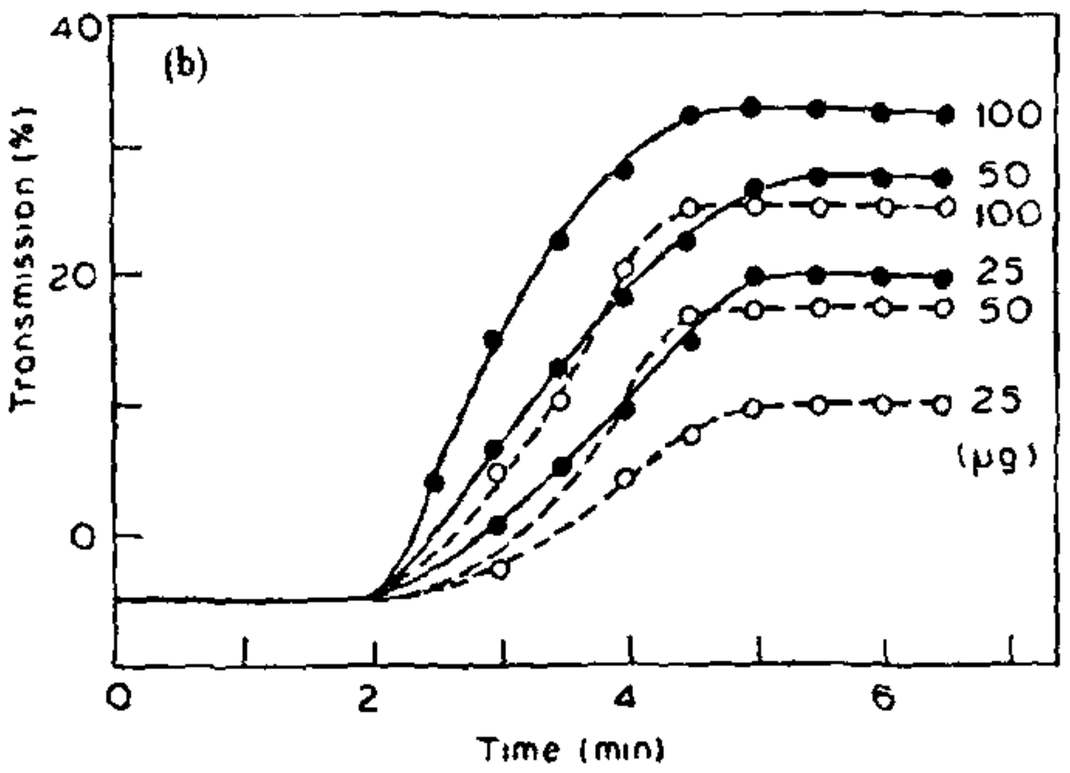


Figure 1a, b. Control (a) and acetylated (b) fish skin collagen-platelet aggregation curves with varying collagen concentration and platelet count: (●) 400,000/mm^3, (○) 200,000/mm^3.

collagen[13]. This may be due to the higher number of basic amino acids particularly epsilon amino group of lysine which play a critical role in platelet aggregation. Acetylated collagen can aggregate the platelets, but less actively. The amplitude of the curve has been considerably reduced and the lag phase also was delayed (figure 1b). The induction of platelet aggregation may be attributed to the triple helical structure and reduction in the platelet-aggregating activity can be due to the loss of epsilon amino groups on the collagen molecule due to acetylation. This clearly suggests that positive amino groups on the collagen molecule might play a critical role in collagen-induced platelet aggregation.

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1. Hugues, J., *Thromb. Diath. Haemorrh.*, 1962, **8**, 241.
2. Hugues, J. and Lapiere, C. M., *Thromb. Diath. Haemorrh.*, 1964, **11**, 327.
3. Zucker, M. B. and Borelli, J., *Proc. Soc. Exp. Biol. Med.*, 1962, **109**, 779.
4. Hovig, T., *Thromb. Diath. Haemorrh.*, 1963, **9**, 264.
5. Zucker, M. B., *Trans. N.Y. Acad. Sci.*, 1974, **36**, 561.
6. Mustard, J. F. and Packham, M. A., *Pharm. Rev.*, 1970, **22**, 97.
7. Fitzsimmons, C. M. and Barnes, M. J., *Thromb. Res.*, 1985, **39**, 523.
8. Hovig, T., *Throm. Diath. Haemorrh.*, 1970, **42**, 137.
9. Chesney, C. M., Harper, E. and Colman, R. W., *J. Clin. Invest.*, 1972, **51**, 2693.
10. Wilner, G. D., Nossel, H. L. and LeRoy, *J. Clin. Invest.*, 1968, **47**, 2616.
11. Wang, C. L., Miyate, T., Weksler, B., Rubin, A. L. and Stenzel, K. H., *Biochim. Biophys. Acta*, 1978, **544**, 555.
12. Vijaya Ramesh, D., Sehgal, P. K. and Dhar, S. C., *Agents Actions*, 1988 (communicated).
13. Vijaya Ramesh, D., Sehgal, P. K. and Dhar, S. C., *Conn. Tiss. Res.*, 1988 (communicated).
14. Kimura, S., Takema, Y. and Kubota, M., *J. Biol. Chem.*, 1981, **256**, 13230.
15. Bose, S. M. and Joseph, K. T., *Arch. Biochem. Biophys.*, 1958, **74**, 46.
16. Born, G. V. R., *Nature* (*London*), 1962, **194**, 927.

STUDIES ON CULTURE PEARL PRODUCTION FROM FRESHWATER MUSSELS

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PEARL, a precious gem is a biological commodity produced by certain bivalve molluscs. China and