Short Paper

Occurrence of Fibronectin in the Plasma of Rainbow Trout

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Mammalian plasma fibronectin (FN) consists of two polypeptide chains which differ slightly in molecular size and are disulfide bonded near their carboxy-termini, and it binds to extracellular matrices such as collagen and heparin.¹⁾ In fish, a plasma FN was found in torpedoid fish *Torpedo californica*²⁾ and carp *Cyprinus carpio*.³⁾ Recently, Takahashi *et al.*⁴⁾ reported that the plasma of rainbow trout *Oncorhynchus mykiss* contained an FN-like protein which was a single polypeptide with 225 kDa and had affinity for heparin and cells, but not for gelatin. In their report, however, there was no evidence for the occurrence of a plasma FN. In the present study, we demonstrate the occurrence of a plasma FN in rainbow trout by means of immunological and affinity chromatographic studies.

Citrated blood and plasma were prepared according to a previous method,⁵⁾ but we substituted anesthetization in 30 ppm of ethyl *p*-aminobenzoate for that in 70 ppm. Immunoblot analysis, sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and carp gelatin affinity column chromatography were performed as described previously.^{5,6)} An anti carp plasma Type I FN (CpFN-I) antiserum, obtained previously,⁶⁾ was absorbed with Sepharose 4B conjugated with a flow-through fraction obtained by the carp gelatin affinity column chromatography of carp plasma, and then it was used as a probe.

Immunoblot analysis (IB-Rp in Fig. 1) showed that unreduced rainbow trout plasma (lane U) contained a main and a minor cross-reacting material with molecular size similar to CpFN-I and carp plasma Type II FN (CpFN-II), respectively. In the reduced condition of rainbow trout plasma (lane R), it showed a single cross-reacting material. The clarified plasma prepared from 60 ml of citrated blood of rainbow trout was applied to a carp gelatin affinity column and then the adsorbed protein was eluted with a linear gradient of 0–1.5 M NaBr/200 ml. As shown in Fig. 1, a single peak component (fraction B, containing 1.1 mg of protein) is observed together with a minor component (fraction A, 0.12 mg) in the shoulder

Fig. 1. Immunoblot analysis of rainbow trout plasma (IB-Rp), elution profile of adsorbed protein in rainbow trout plasma on a carp gelatin affinity column, and SDS-PAGE of fractions A and B.

preceding the peak. Polypeptide compositions in both fractions were analyzed by SDS-PAGE (Fig. 1). The unreduced fraction B contained only a single chain with more than 400 kDa (lane B), which was changed by reduction with 2-mercaptoethanol to a single polypeptide with about 230 kDa (lane C). On the other hand, the unreduced fraction A contained mainly a polypeptide chain with about 230 kDa (lane A), and its mobility did not change by reduction (data not shown). These results indicated that fraction B contained a typical plasma FN, consisting of subunits with about 230 kDa that were linked by disulfide bonds into dimers, and possessing cross-reactivity to anti CpFN-I antiserum and gelatin-binding capacity. Furthermore, it was found that the plasma seemed to contain the other type of FN (fraction A) similar in molecular properties to CpFN-II.

References

- 1) K. M. Yamada: *Ann. Rev. Biochem.*, **52**, 761–799 (1983).
- 2) E. Engvall, E. Ruoslahti, and E. J. Miller: *J. Exp. Med.*, **147**, 1584–1594 (1978).
- 3) N. Uchida, M. Watanabe, M. Kakiuchi, H. Anzai, and E. Nishide: *Nippon Suisan Gakkaishi*, **56**, 315–322 (1990).
- 4) K. Takahashi, T. Suzuki, and K. Mori: *Nippon Suisan Gakkaishi*, **58**, 343–347 (1992).
- 5) N. Uchida, T. Suzuki, S. Yotsuji, H. Anzai, and E. Nishide: *Bull. Coll. Agr. & Vet. Med., Nihon Univ.*, **47**, 178–183 (1990).
- 6) N. Uchida, M. Watanabe, M. Kakiuchi, H. Anzai, and E. Nishide: *Nippon Suisan Gakkaishi*, **58**, 1107–1113 (1992).

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