Multimeric Composition of Endothelial Cell-Derived von Willebrand Factor

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The multimeric composition of human endothelial cell (EC)-derived von Willebrand factor (vWF) was studied using SDS-agarose gel electrophoresis and autoradiography. Two multimers were found in lysates prepared from confluent cultures of human umbilical vein endothelial cells. The smaller multimer had a molecular weight (mol wt) of approximately 950 Kd, while the second was larger than those seen in plasma. When electrophoresis was performed using the discontinuous buffer system of Ruggeri and Zimmerman, the small multimer consisted of a single band migrating with the slowest-moving component of the corresponding plasma triplet. The large EC-vWF multimer was detected in culture media conditioned with EC monolayers for ten minutes. It remained the only multimer in media conditioned for up to three days. Calcium ionophore A23187 increased the amount of the vWF multimer released into the culture media, but did not change its multimeric composition. The small multimer was never detected in the EC-conditioned media. These findings suggest that (1) a large, fully polymerized multimer of vWF is released from the ECs, while the small multimer probably represents a major intermediate component in the process of multimerization, and (2) plasma vWF multimers are probably generated from the large endothelial vWF after it is released into the circulation.

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Fig 1. (A) Autoradiographic patterns of vWF multimers separated by SDS-1.5 g% glyoxyl agarose gel electrophoresis. EC, endothelial cell lysate; NP, normal plasma. Lysate of cultured endothelial cells was diluted 1:5 (vol/vol) and made to contain 1 g% SDS and 30 mmol/L iodoacetamide. Normal plasma was diluted 1:15 with sample buffer. Fifteen liters of each sample was loaded onto the gel. (B) Autoradiographic patterns of vWF multimers separated by SDS-1.5 g% low-gelling-temperature agarose gel electrophoresis using the discontinuous buffer system. Sample treatment was the same as in (A).

Multimeric composition of vWF secreted from cultured ECs. vWF in the SFM conditioned with EC monolayers for 60 minutes (Fig 3A) consisted of a single multimer corresponding to the high mol wt form in EC lysates (Fig 3C). This large form was detected in media conditioned with ECs for as brief as ten minutes. Its concentration in the media increased with further incubation, but it still remained the only multimer after conditioning for up to four hours (data not shown). When endothelial monolayers were incubated in SFM longer than four hours, the ECs detached and degeneration became apparent. Addition of A23187 increased the vWF antigen concentration in conditioned media but did not change the multimeric pattern (Fig 3B). Similarly, when SM was conditioned with ECs for 60 minutes and 24 hours (Fig 3, E and F), only the very high mol wt form of vWF appeared. The concentrations of vWF multimers pre-existing in the serum medium (Fig 3D) remained essentially unchanged. The same result was also obtained when the conditioning with serum medium lasted up to three days (data not shown).

DISCUSSION

The studies reported here demonstrate that vWF contained in confluent cultured human umbilical vein endothelial cells consists of two multimers detectable by SDS-agarose gel electrophoresis. The first multimer, with a mol wt estimated at 950 Kd, migrated along with the second-smallest vWF multimer in normal plasma. However, this component was different from plasma vWF in that it consisted of only one, instead of three bands as shown in the high-resolution agarose gels. This small multimer was not released from the cultured endothelial cells in the absence of
cellular disruption. In contrast, the second multimer (larger-than-normal plasma vWF multimers) was readily released by the endothelial cells into the culture media with or without A23187 stimulation. These findings suggest that the two multimers are functionally different and that only the fully polymerized high mol wt form of vWF is available for release. The function of the smaller multimer is unclear. It may represent an intermediate molecule in the process of multimerization.

Previous studies have demonstrated that endothelial cells synthesized and released very high mol wt forms of vWF. However, these studies also showed the concurrent secretion of the vWF multimers found in normal plasma. One group of investigators has reported that endothelial cells synthesized and released predominantly low mol wt forms of vWF. More recently, this same group has reported that vWF in cultured endothelial cells consisted predominantly of high and low mol wt forms, with very little of the intermediate sizes. In order to explain these findings, two secretory pathways were postulated: (1) a constitutive pathway in which predominantly dimeric forms of vWF were secreted, and (2) an inducible pathway in which very high mol wt forms of vWF were released in response to calcium ionophore A23187 or thrombin. In the present studies, the low mol wt form of EC-vWF comigrated with IgM and was, therefore, tetrameric. It was never found in the endothelial cell culture media. The high mol wt form was found in the culture media with and without stimulation by A23187. Thus, it is unnecessary to postulate two discrete secretory pathways for the release of vWF from endothelial cells. However, these results do not exclude the existence of multiple pathways.

The reasons for the apparently conflicting results regarding the multimeric composition of vWF in, and released from, endothelial cells are not clear. Different experimental approaches and conditions may account for the varied results reported in the literature. In the present study, instead of using metabolic labeling and immunopurification, the conditioned culture media or the lysates were incubated with sample buffer containing SDS and iodoacetamide, and subjected to electrophoresis without prior immunoprecipitation. The vWF multimeric pattern was then visualized after probing with radiolabeled antibody. Furthermore, the EC monolayers were incubated in SFM for less than four hours rather than for days, since we have found that endothelial cells began to detach after four to six hours in the SFM. If, however, we were able to obtain similar results (i.e., secretion of only the high mol wt form of vWF) in the SM conditioned for up to three days with endothelial cells.

The results reported here, demonstrating the secretion of only an extra-large form of vWF by endothelial cells, suggest that plasma vWF multimers are generated after the release of vWF into the circulation. Further studies are being undertaken to elucidate the mechanisms responsible for conversion of endothelial cell vWF into the plasma pattern.

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