RAPID COMMUNICATION

Multimerin: A Series of Large Disulfide-Linked Multimeric Proteins Within Platelets

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Platelets contain proteins with biochemical properties that are well adapted to promoting hemostasis. One important adhesive protein is von Willebrand factor (vWF), which is a very large protein comprised of a series of multimers, ranging from 860,000 to over 10 million daltons. In this report we describe a second platelet protein, p-155, which has a similar unique multimeric composition. Using agarose-acrylamide gel electrophoresis, platelet p-155 was shown to be composed of multimers ranging from less than 450 Kd to many million daltons. Based on this unique structure, we propose that the native molecule be designated as multimerin. Comparison with vWF showed that multimerin contained less of the very high molecular weight multimers. Differential reduction demonstrated that the smallest multimer is a trimer, composed of three 155-Kd subunits. Platelet releasate was demonstrated to contain mainly the smaller multimers, suggesting that the larger multimers bind to the platelet surface. Other studies indicate that multimerin and vWF are the two largest platelet proteins and the only two platelet proteins exhibiting a complex, disulfide-linked multimeric composition with variability in multimer size.

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2556
Immunoblotting was performed as described using primary antibodies JS-1 (monoclonal anti-p-155), rabbit anti-p-155, rabbit anti-vWF (Dako Corporation, Carpinteria, CA). CH-1 (monoclonal anti-thrombospondin), and alkaline phosphatase-conjugated anti-human IgM (Jackson ImmunoResearch, Biocan Scientific Inc, Mississauga, Ontario, Canada). The binding of the primary antibody to the transblotted protein was detected using alkaline phosphatase-conjugated goat antimouse IgG or goat antirabbit IgG (Jackson ImmunoResearch).

Silver staining was used to detect platelet proteins separated by nonreduced agarose/acylamide gel electrophoresis followed by reduced SDS-PAGE.

RESULTS

Investigation of the native structure of multimerin by agarose/acylamide gel electrophoresis demonstrated the complex multimeric structure of this protein. Analysis of the nonreduced multimerin in platelet lysate by agarose/acylamide gel electrophoresis followed by immunoblotting demonstrated discrete multimers of varying size (Figs 1 and 2). When analyzed by a 1.5% acrylamide/1.25% agarose gel, the smallest multimer of multimerin was demonstrated to migrate faster than thrombospondin (Mr 450 Kd, nonreduced) (Fig 2). Under the same conditions, the second multimer migrated at a similar position to IgM. The majority of the multimers had a slower migration than IgM. Comparison with vWF in the same platelet lysate demonstrated differences both in multimer spacing and in the range of size distribution. The multimers were spaced closer than those of vWF and the multimers of multimerin tended to be smaller than the multimers of vWF. We were unable to assign specific molecular weights to the individual multimers because the R<sub>c</sub> plots were not linear. The different migration positions of vWF and multimerin multimers confirm the unique identity of these two proteins.

The size of the intracellular multimerin was compared with the released protein. Platelets were lysed in the presence of proteolytic inhibitors. Other platelets were activated with thrombin and the releasate treated with the same proteolytic inhibitors. We have previously demonstrated that this does not alter the size of the p-155 subunit. The releasate contained more of the smaller multimers than platelet lysate (Fig 2). Multimers on the platelet surface were assessed by immunoprecipitation of surface 125<sup>I</sup>-labeled, thrombin-activated platelets followed by agarose/acylamide gel electrophoresis and autoradiography (Fig 3A, top). Multimerin expressed on the platelet surface showed a similar multimer pattern compared with the whole platelet lysate (Fig 1).

We have previously used differential reduction and SDS-PAGE to demonstrate variability in the native molecular weight of p-155. Most of the reduced protein is the 155-Kd subunit, but there is a small amount of a 170-Kd subunit (Fig 4). This suggests that partial reduction should generate three different sizes of dimers arising from the three combinations of these two subunits. Using conditions of increasing reduction, three intermediate bands were noted that were larger than the fully reduced p-155 protein but were smaller than the smallest nonreduced multimer. These intermediate bands were not evident under nonreducing conditions (Fig 4, upper panel). Full reduction of the three bands generated 170-Kd bands, 155- and 170-Kd bands, and 155-Kd bands, respectively (Fig 4, lower panel). The band at 200 Kd (Fig 4, upper panel, lanes A and B) was not seen consistently in p-155 immunoprecipitates and was not identified as a component of the individual multimers (Fig 3A). These results indicate that the smallest multimer of multimerin is a trimer. This conclusion is also supported by the demonstration that the smallest multimerin multi-
mer migrates faster than thrombospondin in a nonreduced gel (Fig 2).

Multimerin immunoprecipitates were subjected to nonreduced agarose/acylamide gel electrophoresis followed by reduced SDS-PAGE. These studies demonstrated that the individual multimers were composed of the 155-Kd subunits (Fig 3A). Immunoblotting using either monoclonal or polyclonal anti-p-155 yielded identical findings. The MoAb JS-1 also identified a smaller amount of a 170-Kd protein in either immunoprecipitates or immunoblots5 (Fig 4). We were unable to demonstrate a selective association of the 170-Kd subunit with any one size of multimer. Similar analysis of vWF (Fig 3B) demonstrated a subunit composition of 220 Kd as previously described.6 Comparison of multimerin with vWF (Fig 1, 2, 3A and B) demonstrated that they differed in subunit size, multimer spacing, and multimer size distribution.

We investigated whether platelets contained any other large proteins using silver staining and nonreduced/reduced separation. Two extremely large, complex multimeric platelet proteins were identified (Fig 3C) corresponding to multimerin and vWF. No other platelet proteins with these migration characteristics were demonstrated. Additionally, no other proteins larger than multimerin or vWF could be identified.

DISCUSSION

The largest platelet protein is vWF, which is composed of disulfide-linked homodimers of 200-Kd subunits.5 Unlike almost all other multimeric proteins, which are composed of a fixed number of subunits linked by disulfide bonds, vWF is a series of multimers that range in size from 860 Kd to as large as 20 million daltons.1 In this report, we describe another extremely large, variably sized, multimeric platelet protein, p-155, that resembles vWF in its complex multimeric composition.

Recently, we reported that p-155 is a soluble platelet protein that is expressed in low numbers on the surface of
RESTING PLATELETS (approximately 600 copies of JS-1 bound per platelet). However, following platelet activation, this protein is released and about 4,000 binding sites for JS-1 are expressed on the platelet surface. Further studies of the p-155 protein produced unexpected results and are the subject of this report. We found that p-155, like vWF, exists as a series of large multimers of increasing size. We suggest that the native protein, which is made of p-155 subunits, should be designated as multimerin, reflecting its multimeric composition.

Using nonreduced/reduced gel electrophoresis we demonstrated that all of the multimers are composed primarily of identical subunits (Fig 3A). Comparison with thrombospondin and IgM indicate that the multimers of p-155 range in size from a trimer of less than 450 Kd to many million daltons. The trimer migrates with an apparent molecular weight that is less than the predicted 465 Kd, based on three 155-Kd subunits. Other multimeric proteins, including thrombospondin, exhibit similar anomalous migration.

Multimerin is released by thrombin and its subunit size is unchanged by platelet activation; however, platelet releasate contains much less of the high molecular weight multimers. The results suggest that the largest multimers of multimerin preferentially bind to the platelet surface. Similar findings have been reported for vWF.

The unexpectedly massive size and unusual multimeric nature of multimerin led us to investigate whether platelets contained other large multimeric proteins besides multimerin and vWF. Platelet lysate was subjected to nonreduced/reduced electrophoresis followed by protein staining. Two large, disulfide-linked multimeric proteins were observed with subunit sizes of 220 Kd (vWF) and 155 Kd (multimerin), respectively. No other large proteins could be identified.

The body contains other extremely large proteins. For example, muscle contains several very large proteins, titin and nebulin. These proteins differ from the largest platelet proteins in that they are not multimeric. The multimeric nature of vWF and multimerin provides multiple functional sites for binding to the platelet surface and for binding to other ligands. Platelet proteins are well adapted to supporting platelet adhesion and aggregation. The contribution of multimerin to these events is under investigation.

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