BRIEF REPORT

Studies on Subcellular Platelet Particles

By Aaron J. Marcus and Dorothea Zucker-Franklin

Under normal physiologic conditions the lipid component of the intrinsic prothrombin activator (blood thromboplastin) is derived from the platelets and is probably available as a lipoprotein complex. However, it is not known whether this complex arises from the platelet membrane, the platelet granules or other cytoplasmic particles. Therefore, an attempt was made to prepare subcellular platelet fractions and correlate clotting activity with morphologic appearance. In addition, the phospholipids of the subcellular particles and their constituent fatty acids and aldehydes were compared to those of the whole platelet.

MATERIALS AND METHODS

Human platelets were processed according to previously reported methods, but with no period of storage. Two Gm. of fresh platelets (derived from 500 ml. of whole blood) were suspended in 30 ml. of 0.25 M sucrose at room temperature. The suspension was vigorously agitated, transferred to an aluminum container, and placed in a -40°C. acetone-dry ice mixture for 5 minutes. The frozen suspension was then thawed at 37°C., shaken, and centrifuged at 1400 g (5°C.) for 10 minutes. The supernatant fluid was set aside and the sediment resuspended in 30 ml. of 0.25 M sucrose and frozen as before. This process was repeated 3 times and the combined supernatants centrifuged again at 10,000 g in 0.88 M sucrose. This step removed the remaining platelet "ghosts." Aliquots of the final supernatant fluid were taken for blood coagulation studies prior to centrifugation at 105,000 g for 1 hour in a Spinco model L preparative ultracentrifuge (40 rotor). Following this period of centrifugation, the material was recovered as a pellet. One hundred such pellets were accumulated, and the lipids were extracted, and then separated by silicic acid column chromatography as previously described. Six hundred mg. of total lipid were obtained from approximately 7 Gm. of pellets which was almost 3 times as much lipid as obtained from whole platelets of comparable weight. The individual phosphatides were studied in the thromboplastin generation test (TGT) and their fatty acid and aldehyde composition was examined by means of gas-liquid chromatography on polar (ethylene glycol adipate) and non-polar (Apiezon L) columns.

The preparative procedures were monitored by phase and electron microscopy. It was found that disrupted platelets could not be adequately visualized in the phase microscope. Mitochondria and alpha granules were indistinguishable and the membranes were not resolved. For electron microscopy, pellets were fixed in 1 per cent osmium tetroxide, dehydrated in increasing concentrations of alcohol and embedded in Epon 812. Thin sections were obtained with an LKB Ultratome and examined with a Siemens Elmskop I electron microscope at magnifications ranging from 5,000-20,000.

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*Wet weight.

†Centrifuge tube shield, 50 ml., Fisher Scientific Co., New York, N. Y.
Fig. 1.—Appearance of sediment removed by centrifugation at 10,000 g. Note platelet "ghosts" and empty platelet membranes (arrow). x17,000.

Fig. 2.—Pellet obtained after centrifugation at 105,000 g in 0.88 M sucrose contains mitochondria (M), alpha granules (G), and membrane fragments (arrow). x30,000.
Table 1.—TGT on Phospholipids from Subcellular Platelet Particles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>und.</td>
<td>11</td>
<td>8</td>
<td>9*</td>
</tr>
<tr>
<td>PS (0.2 mg.)</td>
<td>1:1</td>
<td>15</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>16</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>PE (0.4 mg.)</td>
<td>und.</td>
<td>38</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>PE (DOC)†</td>
<td>und.</td>
<td>&gt;90</td>
<td>77</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>1:1</td>
<td>&gt;90</td>
<td>&gt;90</td>
<td>85</td>
</tr>
</tbody>
</table>

*Substrate clotting time in seconds.
†Solubilized with sodium desoxycholate.

Table 2.—Fatty Acids of Subcellular Platelet Particles (moles per cent)

<table>
<thead>
<tr>
<th>Familiar Name</th>
<th>Shorthand Designation</th>
<th>Ethanolamine Phosphoglycerides</th>
<th>Serine Phosphoglycerides</th>
<th>Lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>16:0</td>
<td>3.8 (3.3)</td>
<td>2.3 (0.7)*</td>
<td>40.4 (33.4)</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1</td>
<td>0.5 (0.3)</td>
<td>0.8 (0.3)</td>
<td>2.0 (0.9)</td>
</tr>
<tr>
<td>Stearic</td>
<td>17:0</td>
<td>0.3 (0.3)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
<td>18:0</td>
<td>12.3 (13.8)</td>
<td>41.0 (47.0)</td>
<td>10.6 (16.5)</td>
</tr>
<tr>
<td>Linoleic and</td>
<td>18:1</td>
<td>9.5 (8.4)</td>
<td>22.2 (22.6)</td>
<td>28.4 (31.3)</td>
</tr>
<tr>
<td>Linolenic</td>
<td>18:2-18:3</td>
<td>1.5 (0.9)</td>
<td>1.7 (0.9)</td>
<td>5.3 (5.2)</td>
</tr>
<tr>
<td>Arachidinic</td>
<td>20:0</td>
<td>0.3</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Arachidonic</td>
<td>20:1</td>
<td>0.3</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>20:2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20:3</td>
<td>1.4 (1.1)</td>
<td>1.5 (0.9)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>20:4</td>
<td>30.4 (37.5)</td>
<td>22.0 (25.0)</td>
<td>9.1 (11.9)</td>
</tr>
<tr>
<td></td>
<td>22:5</td>
<td>5.04 (0.5)</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>22:6</td>
<td>2.2 (0.6)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22:un†</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Whole platelet.
†Unsaturated.

Table 3.—Fatty Aldehydes of Subcellular Platelet Particles (moles per cent)

<table>
<thead>
<tr>
<th>Familiar Name</th>
<th>Shorthand Designation</th>
<th>Ethanolamine Phosphoglycerides</th>
<th>Serine Phosphoglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitaldehyde</td>
<td>16:0</td>
<td>9.4 (10.5)*</td>
<td>2.0 (0.3)*</td>
</tr>
<tr>
<td></td>
<td>17:0 br†</td>
<td>0.8 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Stearaldehyde</td>
<td>18:0</td>
<td>15.3 (19.0)</td>
<td>2.0 (0.2)</td>
</tr>
<tr>
<td>Oleylaldehyde</td>
<td>18:1</td>
<td>5.6 (3.8)</td>
<td></td>
</tr>
</tbody>
</table>

*Whole platelet.
†Branched chain.

Results and Discussion

Electron microscopy revealed that despite the freezing and thawing process a large number of platelet "ghosts" consisting of intact membranes, vacuoles, swollen mitochondria, and occasional granules were present (fig. 1). These were removed by centrifugation at 10,000 g in 0.88 M sucrose. Subsequent pellets contained mitochondria, alpha granules, glycogen, and small pieces of membranes varying from 0.1 μ to a few thousand A in length
(fig. 2). When this material was tested as platelet reagent in the TGT, the substrate clotting times were equal to those of whole platelet and brain cephalin. The platelet "ghosts" and amorphous material, which were removed by preliminary centrifugation, showed some activity, but at no concentration were they comparable to levels achieved with the pellet material.

Coagulation studies on the separated ethanolamine (PE) and serine (PS) phosphoglycerides were similar to those previously observed with whole platelets and brain.\(^1\) PS promoted clotting activity in the TGT whereas PE did not. This is shown in table 1. On a dry weight basis, the lipid requirement for clotting activity was the same with subcellular PS as it was with whole platelet PS.

Tables 2 and 3 show the fatty acid and aldehyde composition of the phosphoglycerides of subcellular platelet particles, expressed as moles per cent. For comparison, the results previously obtained with phosphatides of the intact platelets\(^1\) are shown in parenthesis. In general, the fatty acid and aldehyde composition of the subcellular particles was similar to the whole platelet. However, there were interesting variations in fatty acids of C\(_{20-22}\) chain length. The particles were characterized by increased amounts of 22:5 and 22:6 fatty acids. These findings were reminiscent of the results reported by Marfarlane, Gray and Wheeldon\(^5\) and Getz et al.\(^6\) where a somewhat similar distribution of long-chain, highly unsaturated fatty acids was found in rat liver mitochondria and microsomes. The results may have a significance in relation to the "availability" of platelet lipid for intrinsic prothrombin activation formation. The presence of an increased number of double bonds enhances the degree of steric hindrance between adjacent fatty chains and results in a lesser degree of Van der Waals attraction.\(^7\) Since platelet phosphatides are probably present as lipoprotein complexes, these would be unstable\(^7,8\) and the platelet lipid (or lipoprotein) would be more readily available for interaction with plasma clotting factors.

**SUMMARY**

The phospholipids of subcellular platelet particles are very similar to those of the intact platelet. However, they contain a higher proportion of long-chain, highly unsaturated fatty acids, which confers upon them physical characteristics suggesting a dynamic biological system.

**SOMMARIO IN INTERLINGUA**

Le phospholipidos de subcellular partículas plachettal es similiissime a illos del plachetta intacte. Tamen, illos contine un plus alte proportion de altemente non-saturate acidos grasse a longe catenas. Isto confere a illos caracteristicas physic que indica possiblemente un dynamic systema biologic.

**REFERENCES**


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