Factors Influencing the Interaction Between Radioactive Iodine and Fibrinogen

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Radioactive iodine is currently used to label proteins as a method of investigating protein metabolism in vivo or as a tracer for studies in vitro. However, recent observations indicate that changes in the molecular characteristics of proteins after iodination may lead to an apparent increase in the turnover rate in vivo (Regoezzi, 1971; Krohn et al., 1972). Incorporation of iodine into proteins has also been used to determine certain characteristics of protein structures (Cohen, 1968). We report here the effect of increased iodination on the properties of fibrinogen in vitro and also the changes in the extent of iodination of subunits of fibrinogen under various conditions.

Human fibrinogen obtained from Abbott Universal Ltd. was used in all experiments. Fibrinogen was iodinated with $^{125}$I by the iodine monochloride method of McFarlane (1963) substituting 0.5, 1.0, 2.0, 4.0, 8.0 and 16 atoms of iodine/molecule of fibrinogen. $^{125}$I was added to each preparation as a radioactive marker and at the end of the reaction free iodide was removed by dialysis. Fibrinogen samples were subsequently dissolved in a mixture containing 4M-urea, 2M-sodium dodecyl sulphate, 0.2M-2-mercaptoethanol and the subunits were separated by sodium dodecyl sulphate–polyacrylamide-gel electrophoresis. The gels were stained and the radioactivity associated with the separated $\alpha$, $\beta$ and $\gamma$ chains was determined in a Packard Autogamma counter. After iodination at pH 7.5, resulting in incorporation of 0.5 atoms of iodine/molecule of fibrinogen, the relative radioactivity associated with the subunits was 2.6 for the $\alpha$ chain and 3.3 for the $\beta$ chain as compared with the radioactivity of the $\gamma$ chain which was used as the reference unit (1.0). With increasing degrees of iodination with $^{125}$I, the radioactivity of the $\gamma$ chain increased proportionately more than that of the $\alpha$ and $\beta$-chains reaching a ratio of 2.0 for the $\alpha$ chain, 2.1 for the $\beta$ chain and 1.0 for the $\gamma$ chain under the conditions of iodination of 16 atoms of iodine/molecule of fibrinogen.

The relative iodination of fibrinogen subunits was also a function of the pH of the iodinating mixture in the range pH 5.5–10.0 studied. At 0.5 atoms of iodine/molecule of fibrinogen, the effect of pH on the relative $^{125}$I content of the subunits is shown in Table 1. Values are expressed as ratios of incorporation of radioactivity into $\alpha$ or $\beta$ chains of fibrinogen/radioactivity incorporated into $\gamma$ chains.

The effect of iodination on the clotting time of the fibrinogen was determined by the addition of thrombin (0.25 N.I.H. units/mg of fibrinogen) to each preparation. The clotting time of the radioactive fibrinogen was calculated as a percentage of that of uniodinated fibrinogen and was found to decrease with increasing degrees of

Table 1. Relative distribution of $^{125}$I in $\alpha$ and $\beta$ chains of fibrinogen as compared with $^{125}$I incorporation into $\gamma$ chains

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>$\alpha/\gamma$</th>
<th>$\beta/\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mm-Tris-maleate</td>
<td>5.5</td>
<td>5.0</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>50mm-Tris-HCl</td>
<td>7.0</td>
<td>2.5</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>2.6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.6</td>
<td>4.2</td>
</tr>
</tbody>
</table>

For details see the text.
iodination reaching 57% of the control at 16 atoms of iodine/molecule of fibrinogen.

The effect of iodination on the ability of fibrinogen to be converted into cross-linked fibrin was investigated in the presence of 10mM-Ca^{2+} and 0.1 ml of fresh human serum. Fibrin was removed after 5 min or 2 h and dissolved in a mixture containing 4.0 M-urea, 2% sodium dodecyl sulphate and 0.2 M-2-mercaptoethanol. The fibrin subunits were separated by sodium dodecyl sulphate-polyacrylamide-gel electrophoresis. The degree of cross-linking was determined by measuring the amount of radioactivity incorporated into the \( \gamma \-\gamma \) chain dimers and \( \alpha \) chain polymers (Gaffney, 1973). Increasing degrees of iodination had no effect on cross-linking.

Our results indicate that the relative incorporation of \(^{125}\text{I}\) into \( \alpha \), \( \beta \) and \( \gamma \) chain sub-units of fibrinogen varies depending on the concentration of iodine and also on the pH values during iodination. The ability to form cross-linked fibrin is unaffected by high degrees of iodination although the clotting time of fibrinogen is significantly decreased at relatively high iodine concentrations in the fibrinogen.


Complexes between Deoxyribonucleic Acid and the Adenosine Triphosphate-Dependent Deoxyribonuclease from Mycobacterium smegmatis

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Complex formation between DNA and the ATP-dependent deoxyribonuclease from *Mycobacterium smegmatis* has been demonstrated (Winder & Sastry, 1971). There is now evidence for more than one type of interaction between DNA and this enzyme and, for convenience, these types of interaction will be referred to as distinct complexes.

We will define complex A as involving that DNA which is subsequently broken down processively by the enzyme, that is, without exchanging with other DNA which may be added subsequent to complex formation. The formation of this complex at low temperature is shown in Fig. 1. The lower line shows the release of acid-soluble products, while the upper line shows the release of acid-soluble material when a large excess of unlabelled DNA was added at the times shown and the reaction was allowed to proceed for a suitable further period. Complex A is represented by the difference between these curves, after correction for the difference in zero-time values. Formation of this complex rigidly requires ATP, and response to varying ATP concentration is similar to that of the overall deoxyribonuclease reaction involving the release of acid-soluble products. The rate of the overall reaction under various conditions appears to be related to the amount of complex A, though the relationship does not appear to be a simple or rigid one and the acid-soluble products released are not all necessarily from complex A.

The amount of DNA which is bound to membrane filters by the enzyme substantially exceeds that in complex A. This is shown for an experiment in which the reaction was limited by the amount of ATP (Table 1). Deproteinization renders this DNA no longer able to bind to the membrane. This excess indicated the formation of a second complex, to be referred to as complex B, which may be defined as that DNA which is bound in ATP-dependent fashion to filters by the enzyme but is not broken down processively by it, though non-processive breakdown may take place.

ATP-independent complex formation between the enzyme and DNA may be referred to as complex C. Formation of such a complex by this enzyme from several bacteria has been shown by ultracentrifugation, phase partition and DNA-cellulose chromatography.

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