A method for the estimation of the activity of the inhibitor of the first component of complement

AM SMITH AND RA THOMPSON

From the Supra Regional Protein Reference Unit, Department of Immunology, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST, UK

SUMMARY The activated first component of complement (C1) possesses an esterase activity in vitro which will hydrolyse an ester of tyrosine to release H+. The activity of the serum inhibitor of C1 esterase may be measured by monitoring its ability to prevent H+ release under standard conditions. This paper describes a method of measuring such activity, monitoring H+ release by the use of either an acid base indicator or pH meter.

C1 esterase inhibitor (C1INH) is an \( \alpha_2 \) glycoprotein, present in serum, which inhibits the effect of the activated first component of complement. Activated C1 possesses an esterase activity and will hydrolyse synthetic esters such as \( N \)-acetyl-\( L \)-tyrosine ethyl ester (ATEE) to produce hydrogen ions. The release of hydrogen ions is inhibited by the presence of C1INH, and this forms the basis for the most commonly used method of measurement of this protein.\(^1\),\(^2\) Its activity can also be measured in a haemolytic system,\(^3\) and the protein can be measured immunochemically using a specific antiserum.\(^4\)

The present method is also based on the inhibition of hydrogen ion release but enables a kinetic analysis of inhibitor activity by the use of either a sensitive pH meter or an acid base indicator.

Methods

REAGENTS


PREPARATION OF C1\(^5\)

20 ml of serum is diluted to 80 ml with distilled water, and the pH is adjusted to 7-4 with 1 M phosphoric acid (\( H_3PO_4 \)). The resultant solution is kept in tubes in ice overnight, and the precipitate formed is spun down and resuspended in saline (8.5 g/l), the volume made up to 80 ml, and the pH adjusted to 5-4 with 0.1 M \( H_3PO_4 \). This solution is again kept in ice overnight, the precipitate formed spun down, the supernatant removed, and the precipitate resuspended in a small volume of saline (8.5 g/l). This suspension of C1 requires activation, by adjustment of pH to 7-2 and incubation at 37°C for 30 minutes, before use in the following assays.

COLORIMETRIC METHOD

The change in absorbance at 434 nm of a bromothymol blue solution is monitored in a dual-beam spectrophotometer (SP1800, Pye Unicam Ltd) linked to a chart recorder. Optical cuvettes of 3 ml volume and 1 cm light path are used. The cell holder is warmed to 37°C. The activity of the C1 preparation alone is first estimated. The following reagents are placed in both blank and reaction cuvettes:

(a) NaCl 8.5 g/l 0.6 ml
(b) bromothymol blue 0.15 g/l 2.2 ml
(c) 0.25 M NaOH approx 0.05 ml

The sodium hydroxide is added to bring the starting pH to 7.2.

The cuvettes are pre-incubated at 37°C. 0.1 ml of the C1 preparation is then added to the reaction cuvette, and 0.1 ml saline to the blank cuvette, followed by 0.1 ml of the 1 M ATEE to each cuvette. The substrate initiates the reaction, and the fall in pH in the reaction cuvette causes increased light absorption at 434 nm, and this is displayed as a sloping line on the chart. The production of acid will cause a decrease in pH of up to 0.1 unit per minute. The reaction is monitored for about 3 minutes, and the final pH will therefore be just below 7.0. The line obtained is straight, demonstrating that the reaction is linear over this pH range. The slope of this line is proportional to C1 activity.

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The analysis of the Cl is repeated using 0.075 and 0.05 ml samples, and a graph of Cl activity vs slope of line is drawn. This should be a straight line through the origin.

To determine the inhibitory effect of serum on the activated Cl, two pre-incubated cuvettes containing reagents a, b, c, as previously, are taken. To both blank and reaction cuvettes 0.025 ml of test serum is added. To the test cuvette alone 0.1 ml of Cl is added and 0.1 ml of saline to the blank cuvettes, and finally 0.1 ml of 1 M ATEE is added to each cuvette as before. Any hydrolysis, as shown by the line on the chart paper, is proportional to the activity of the portion of the Cl not inactivated by the test serum. The degree of inhibition may be deduced from Figure 1.

The inhibitory capacity is thus measured in µl of Cl. A standard serum is used and designated 100%, other test sera being reported as a percentage of the standard. The standard serum comprises sera obtained from the Blood Transfusion Service. The pool is aliquoted in 1-ml units and stored at −70°C until used. Each aliquot is discarded after use. Specimens that show a low inhibitory capacity are reanalysed using larger sample volumes, such that the total inhibitory capacity is approximately 100% of those of the standard serum.

**USE OF pH METER**

The change in pH is monitored using a sensitive pH meter and electrode (PW9409 Digital pH meter and compound silver chloride electrode, Pye Unicam Ltd) linked to a chart recorder. The procedure is essentially the same as for the colorimetric method except that the dye solution is replaced by the same volume of distilled water. The reaction is carried out in the test tubes in a waterbath at 37°C. With a single electrode a simultaneous blank cannot be used and a separate estimation must be performed, omitting the Cl.

Traces are produced on the chart recorder in a manner analogous to the colorimetric method, and results are calculated in an identical fashion.

In the absence of a chart recorder the rate of reactions may be obtained by use of a digital pH meter.

**IMMUNOCHEMICAL ESTIMATION**

Immunochromatographic analyses are carried out using radial immunodiffusion. The antisera used was raised in sheep against purified human ClINH and absorbed with serum of a CIINH deficient patient to render it monospecific.

**Results**

Figure 2 shows tracings of change in pH using the colorimetric method produced by 0.1 ml of the Cl preparation in conjunction with (a) 0.1 ml NaCl (8.5 g/l); (b) 0.1 ml normal serum; (c) 0.1 ml serum from a patient with clinical hereditary angioneurotic oedema.

The mean (±1 SD) inhibitory capacity obtained in 20 healthy laboratory staff is 99 (±13) expressed as a percentage of the standard serum. The between-batch coefficient of variation for the colorimetric method is 12.9% at a mean level of inhibition of 97.2% of the standard serum. Using a pH meter, the between-batch coefficient of variation is 9.6% at a mean level of inhibition of 98.5% of the standard serum. Analysis of 100 specimens was undertaken by both methods. The linear regression is 0.85 with a slope of 0.87.

Specimens for this analysis are frequently received through the post with resultant varying time intervals between venesection and testing. Figure 3
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Fig. 2 Specimen tracings of C1INH activity.

(a) NaCl  Slope = 2.0  (b) Normal serum  Slope = 1.0  (c) Serum from patient with angioneurotic-oedema

Fig. 3 C1INH—comparison of immunochemical levels and functional activity. ***Patients with hereditary angioneurotic oedema due to C1INH deficiency. ▲Patients in whom the functional levels of C1INH were found to be low, but levels in a fresh serum specimen were found to be normal.

shows the comparison of C1INH functional activity, by the colorimetric method, with immunochemical level in 117 sera from patients. The correlation coefficient is 0.61. The crosses signify patients with known hereditary angioneurotic oedema due to C1INH deficiency. A number of sera (▲ in Fig. 3) initially showed functional levels considerably lower than the immunochemical values. However, when freshly obtained specimens were checked, normal functional and immunochemical levels were found in each case. It was observed that sera stored at room temperature for more than 24 hours lost some C1INH activity, and in certain pathological sera this was exaggerated, causing artefactually low functional levels in comparison with immunochemical measurements; this is being further investigated. As yet, no specimen analysed when freshly obtained has shown the reported pattern of
normal immunochemical level with absent functional activity.

Discussion

Most current methods for the estimation of C1INH utilise the acidometric titration principle, and some recommend an automatic apparatus which is designed specifically for such titration. Such titrations may take up to half-an-hour for the evaluation of a specimen containing normal amounts of C1INH. The method described here uses the same basic reaction as previous methods but uses non-specialised equipment which should be readily available in a well-equipped hospital laboratory. It relies on a one-stage addition, rather than incremental additions of reagent, and is therefore more rapid in use. Further, using the equipment described, the reaction may be monitored throughout its course, and linearity verified. Some serum samples show abnormally high rates of decay of functional C1INH activity, and specimens for this analysis should be stored at −70°C. If such storage is not possible then low values should be treated with caution and should be checked on a freshly obtained specimen.

References


Requests for reprints to: Mr AM Smith, Supra Regional Protein Reference Unit, East Birmingham Hospital, Department of Immunology, Bordesley Green East, Birmingham B9 5ST, UK.
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A M Smith and R A Thompson

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