A microassay for protein determination using microwaves

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Recent developments in biochemistry demand faster screening methods which can work with small volumes. This is equally true for estimation of proteins as well. Akins and Tuan [1] have suggested the use of microwaves for enhancing the sensitivity of protein estimation by Lowry’s method [2]. Young et al. [3] have described an automated colorimetric microassay system for protein estimation using an ELISA plate reader. In the present work, we describe a method which is based upon synergy of the above two approaches. The result is a fast and sensitive protocol which can work with small volumes.

Materials and methods

Materials. All chemicals were of analytical grade. Soybean trypsin inhibitor, α-chymotrypsin, ribonuclease, and trypsin were purchased from Sigma Chemical (St. Louis, MO). Bovine serum albumin was procured from Merck-Schuchardt (Germany); lysozyme (egg white) and goat IgG were from Bangalore Genei, India. Folin-Ciocalteau’s reagent was obtained from Sisco Research Laboratories (Mumbai, India).

Method. Protein solution (5 ll) and Triton X-100 (35 ll of 0.05%) were transferred to the wells of 96-well flat-bottom microtitre plate. The reaction mixture was incubated with 200 ll of reagent solution, which was prepared by mixing reagents A and B in the ratio of 50:1 (reagent A, 2% sodium carbonate in 0.1 N NaOH; reagent B, 0.5% copper sulfate in 1% sodium tartarate). After 10 min, 20 ll of 1 N Folin’s reagent (diluted at the ratio of 1:1 with distilled water) was added. The solutions were mixed by a gentle shake of the plate. After this, the microtitre plate was kept in a domestic microwave oven along with a beaker containing a volume of water sufficient to make the total volume of the liquid in the chamber as 100 ml. This was done to avoid overheating of the samples. The plate was read at 700 nm after irradiating for 20 s at a frequency of 2.45 GHz.

Concentration limits for the presence of interfering substances. The effects of the presence of various substances, viz., buffer salts, reductants, EDTA, and urea were studied. The assay was performed in the same manner as above except that the protein solutions were prepared in distilled water containing the above-mentioned components.

Results and discussion

The assay described here is suitable for all situations in which standard assays are currently used. The ability to determine protein concentration accurately and rapidly facilitates process development in which protein determination is required for multiple samples or at multiple steps.

Fig. 1 shows the correlation between the absorbance obtained at 700 nm and the concentration of BSA added to each well. This curve is linear from 2.5 to 200 lg/ml.

Fig. 2 shows the response of different proteins in the same assay. This differential response toward different proteins is similar to what is observed in currently used protocols [4] for Lowry’s method [2].

Table 1 shows that the microwave microassay tolerates the presence of higher concentrations (compared to present protocols) of various substances which are known to interfere with Lowry’s method. This obviates the need for using special extra steps to take care of interfering substances [5,6].

The present work has been carried out using a 20-L domestic oven at 750 W with cavity dimensions of 300 x 290 x 210 mm. Akins and Tuan [1] have provided a very useful discussion on the selection of microwave
Table 1

Effects of various reagents and buffers on the Lowry's microassay of bovine serum albumin

<table>
<thead>
<tr>
<th>Substance</th>
<th>Standard assay</th>
<th>Standard microassay</th>
<th>Microassay using microwave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>20 mM</td>
<td>30 mM</td>
<td>30 mM</td>
</tr>
<tr>
<td>Citrate</td>
<td>3.5 mM</td>
<td>1 mM</td>
<td>5 mM</td>
</tr>
<tr>
<td>Glycine</td>
<td>1mM</td>
<td>M5 mM</td>
<td>15mM</td>
</tr>
<tr>
<td>Phosphate</td>
<td>200 mM</td>
<td>200 mM</td>
<td>200 mM</td>
</tr>
<tr>
<td>Tris</td>
<td>2.5 mM</td>
<td>25 mM</td>
<td>400 mM</td>
</tr>
<tr>
<td>Reductant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>1.5 uM</td>
<td>MmM</td>
<td>mM</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>150 uM</td>
<td>100uM</td>
<td>150 uM</td>
</tr>
<tr>
<td>Urea</td>
<td>150 mM</td>
<td>4 M</td>
<td>400 mM</td>
</tr>
</tbody>
</table>

Note: The assay was performed with the protein solutions prepared in distilled water containing the various components. The numbers give the maximum possible concentrations of the substances which affect the color development.

Ovens. In their experience, several microwave ovens of different brands and cavity sizes worked equally well. Most of the modern domestic microwave ovens provide reasonably uniform irradiation within the oven chamber. Fine control of irradiation time is obviously a crucial desirable feature for the microwave oven being used in these experiments. Like any other conventional method, the user should draw a standard curve and adjust exposure time by a few seconds if necessary. It is important that the total amount of fluid (sample and additional water) is kept constant during various assays which are being compared.

Many authors [7-10] have discussed the mechanism of the reduced reaction time while using microwaves. Polar molecules like water align with the continuously changing electromagnetic field generated by microwaves. The rotational energy of water is then dissipated as heat. Domestic microwave ovens use 2.45 GHz frequency. As macromolecules like proteins cannot reorient at this frequency fast enough, these do not spin.

It is debatable whether the effect of microwaves in assisting chemical reactions is limited to thermal effects. Caddick [11] provides a discussion which reflects the confusion in the literature over this point. Akins and Tuan [1] have described controls which indicate that the reduced color generation time in Lowry's assay is not solely due to the thermal effect.

Finally, use of more sophisticated microwaves with noncontact infrared sensor feedback temperature control has shown that even when temperature is not allowed to rise, microwaves do accelerate the reaction rates [11,12].

To conclude, the advantages of the present protocol are:

- Small volumes (up to 5 lL) can be handled.
- The assay time is reduced to 20 s from the normal 30-min time [2].
- As the assay is carried out using an ELISA plate reader, automation is possible.

Thus, the method can be adapted to a high-throughput screening plateform.

Acknowledgments

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References