RADIOMETRIC METHOD OF DETERMINATION OF GLUCOSE-6-PHOSPHATASE ACTIVITY

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UDC 577.158.347.084:543.52

A simple and reliable radiometric method of determination of glucose-6-phosphatase activity is described. The method is based on formation of C^{14}-glucose as a result of the action of the enzyme on C^{14}-glucose-6-phosphate.

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In this paper we describe a simple and reliable radiometric method of determination of glucose-6-phosphatase (G-6-Pase) (D-glucose-6-phosphate phosphohydrolase, ICE 1.3.9) [1]. The principle of the method is that by the action of the enzyme on C^{14}-glucose-6-phosphate (C^{14}-G-6-P) C^{14}-glucose is formed. The C^{14}-G-6-P remaining undecomposed is removed from the medium as the barium salt. By comparing the radioactivity of the supernatant from the experimental sample with the activity of C^{14}-glucose equimolecular to the amount of C^{14}-G-6-P * before the reaction, the amount of C^{14}-G-6-P decomposed by the enzyme and, hence, the activity of G-6-Pase, can be calculated. For the enzyme reaction low concentrations of C^{14}-G-6-P were used in which the enzyme reaction in practice obeys a first-order equation. If the substrate concentration is expressed as a percentage of its initial concentration, the first-order equation [4] has the form:

$$k_t = 2.3 \lg 100/(100-X), \quad (1)$$

where X is the concentration of the converted part of the substrate.

The unit of G-6-Pase activity is taken as the amount of enzyme which, under certain conditions (2.8 μmole C^{14}-G-6-P/ml; pH 6.5; t 37°) decomposes 50% of the C^{14}-G-6-P in 10 min. The unit of activity corresponds to a velocity constant of 0.0692 min^{-1}.

Since the value of k' is directly proportional to the enzyme concentration, activity of the enzyme can be estimated by comparing k' of the unknown enzyme preparation with k' for the unit [4]:

$$\frac{k'(\text{measured})}{k'(\text{for one unit})} = \text{number of units of enzyme} \quad (2)$$

Preparation of C^{14}-G-6-P. The following reagents are introduced into a test tube: 1 ml C^{14}-glucose† solution containing 50 μmoles of this substance with a total activity of 6 μCi, 1 ml $MgCl_2$ solution containing 25 μmoles, 1 ml ATP solution (preliminarily neutralized), containing 60 μmoles, 0.2 M borate buffer (pH 7.4), and 1 ml hexokinase‡ (5 mg hexokinase dissolved in 1 ml 0.2 M borate buffer, pH 7.4). After mixing, the tube and its contents are placed in a water thermostat (37°) and incubated for 1 h, then transferred for 2 min into a boiling water bath, and then rapidly cooled. The coagulating hexokinase is removed by filtration. The resulting solution contains from 91.2 to 95% (mean 92.8 ±1.4%) of the C^{14}-G-6-P (relative to the amount of C^{14}-glucose).

Preparation of a Standard Solution of C^{14}-glucose. The standard solution of C^{14}-glucose is prepared in the same way as the solution of C^{14}-G-6-P, but has zero incubation time with hexokinase. The volume of C^{14}-G-6-P solution and of the standard solution of C^{14}-glucose is made up as required.

*In addition, the C^{14}-glucose must have the same specific activity per micromole as the C^{14}-G-6-P.

†In our experiments we used a Soviet preparation of 1-C^{14}-glucose.

‡Crystalline hexokinase from the firm "Cyclo" (USA).

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TABLE 1. Ratio between Reaction Constants for Various Dilutions of G-6-Pase

Activity of standard (pulses/min)	Activity k' (pulses/min)	Activity k'_2 (pulses/min)	Activity k'_4 (pulses/min)	k'	k'_2	k'_4	$\frac{k'}{k'_2}$	$\frac{k'_2}{k'_4}$	$\frac{k'}{k'_4}$
2325	643	305	149	0,030	0,014	0,0069	2,13	2,02	4,34
2396	705	390	218	0,035	0,018	0,009	1,95	2,00	3,90
2396	731	434	225	0,036	0,019	0,009	1,90	2,10	4,00
2618	822	513	272	0,038	0,020	0,010	1,90	2,00	3,80
2240	750	447	210	0,040	0,020	0,010	2,00	2,00	4,00
2240	724	400	209	0,040	0,019	0,010	2,10	1,90	4,00
2240	764	399	233	0,041	0,019	0,010	2,15	1,90	4,10
2325	857	437	313	0,045	0,022	0,012	2,04	1,89	3,79
2325	880	447	262	0,046	0,022	0,011	2,09	2,00	4,18
1819	712	408	210	0,050	0,026	0,012	1,92	2,16	4,16
1819	712	415	240	0,050	0,026	0,013	1,92	2,00	3,85
1932	839	522	322	0,058	0,030	0,016	1,93	1,89	3,65
2240	1009	636	316	0,060	0,031	0,015	1,93	2,07	4,00
2696	1249	620	405	0,060	0,028	0,015	2,14	1,89	4,00
2612	1176	742	372	0,060	0,032	0,015	1,89	2,13	4,00
2696	1250	734	398	0,061	0,031	0,015	1,96	2,07	4,07
2612	1216	734	379	0,064	0,032	0,016	2,00	2,00	4,00

Course of the Determination. Into a centrifuge tube (volume 10 ml) are poured 0.5 ml maleate buffer (pH 6.5) [3] and 0.3 ml of the prepared solution of C^{14}-G-6-P (0.36 μCi and the sample is kept in a water thermostat (37°) for 5 min. Next, 0.2 ml of 15% liver tissue homogenate * is added. After 10 min, 1.5 ml 5% $ZnSO_4$ and 1.5 ml 0.3 N $Ba(OH)_2$ † are added, and the sample, after standing for 20 min, is centrifuged at 3000 rpm for 15 min. The supernatant is drawn off and applied in a volume of 0.1 ml to a target (diameter 16 mm). Solution from one sample is applied to three targets which are dried in the thermostat at 75° for 2 h. A second parallel sample is treated in the same way. Standard samples differ from experimental in that, instead of C^{14}-G-6-P, they contain standard solution of C^{14}-glucose. Besides the experimental and standard samples, controls must also be set up (control for C^{14}-G-6-P content), differing from the experimental samples in that they have zero incubation time with G-6-Pase. The radioactivity of the standard, experimental, and control samples is determined in the course of 3 min on a T-25 BFL end-type counter (thickness of mica 1 mg/cm^2), connected to a B-2 radiometer. The mean reading of the control samples is subtracted from the values of the mean activity of the standard and experimental samples, after which the quantity of substrate not decomposed by the enzyme (100-X) and the value of k' are calculated from formula (1).

Trials of the Method. The results of investigations showed that in the C^{14}-G-6-P concentration range from $2.8 \cdot 10^{-3}$ to $0.89 \cdot 10^{-3}$ M the enzyme reaction obeys a first-order equation. Since the value of k' is proportional to enzyme concentration, the method was tested by comparing the constants of the enzyme preparations (k') when the enzyme was diluted twice (k'_2) and 4 times (k'_4). The C^{14}-G-6-P and G-6-Pase were incubated for 10 min at 37°. The results of our determinations of the ratio between the constants (Table 1) show that the experimental value of $k' : k'_2$ was 1.99 ± 0.09, or 99.5 ± 4.5% of the theoretical value obtained by Eq. (2), and the ratio $k'_2 : k'_4$ was 2 ± 0.08 (100 ± 4%), and the ratio $k' : k'_4$ was 3.99 ± 0.16, or 99.7 ± 4% of the theoretical value (4). Our radiometric method of measuring activity of G-6-Pase suggests that this principle might profitably be employed in the measurement of alkaline and acid phosphates activity by the same method.

LITERATURE CITED

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*In our experiments the G-6-Pase was obtained from rat liver homogenates made up in maleate buffer (pH 6.5).

†The $ZnSO_4$ and $Ba(OH)_2$ solutions must be suitably matched [2].