THE METALLOENZYME NATURE OF CALF THYMUS DEOXYNUCLEOTIDYL TRANSFERASE

E. SABBIONI

Chemistry Division, Euratom Joint Research Center, 21020 Ispra, Varese, Italy

Received 12 October 1976

1. Introduction

Calf thymus gland is a source of different deoxynucleotide polymerizing enzymes including terminal deoxynucleotidyl transferase which catalyzes polymerization of deoxynucleoside triphosphate [1] (E.C. 2.7.7.X).

Although the biochemical role of terminal transferase is not known, a possible relation to DNA polymerase has been suggested [2] in agreement with some observations on the effect of Zn deficiency on the formation of DNA polymerase [3].

Terminal transferase is an Mg activated enzyme [4] but it has been suggested that the protein utilizes also a tightly bound cation in its catalysis [5]. Since Zn has been found as cofactor of other transferase enzymes such as *E.coli* and sea urchin DNA polymerase [6,7] DNA dependent T7 RNA polymerase [8] and reverse transcriptase from mammalian RNA type C viruses [9], we have investigated whether the calf thymus terminal transferase is a metalloenzyme. The results of the present paper show that the protein is effectively a Zn-enzyme.

2. Materials and methods

Metal free buffers were prepared in quartz bidistilled water that had been passed repeatedly through Chelex 100 columns at slow flow rates. All glass- and plastic-ware used were acid-washed.

Carrier free ^{65}Zn was prepared by proton activation of Cu foil at the cyclotron of Milan University [10].

^{65}Zn radioactivity was measured by γ-ray spectrometry using a Ge(Li) detector or Cerenkov spectroscopy [11].

Microdetermination of protein in the eluate from Sephadex G-25 was carried out by a highly sensitive radiobiuret method [11].

2.1. *Preparation of terminal transferase and apoenzyme*

Terminal transferase from calf thymus gland was prepared according to the procedure of Yoneda and Bollum [1] by chromatography on phospho-cellulose, DEAE–cellulose, hydroxylapatite and Sephadex G-100 with the modification of [5].

The specific activities of two different preparations were 13 400 and 16 100 units (one unit catalyzes the incorporation of 1 nmol of deoxydenylate into an acid soluble product per hour, 37°C, pH = 6.8 with $d(pT)_6$ as primer).

The preparations were judged homogeneous as determined by polyacrylamide gel electrophoresis.

The apoenzyme was prepared by incubating holoenzyme with 5 mM EDTA in cacodylate buffer, pH = 6.8 at 25°C for 30 min and the solution was dialyzed at 4°C against several changes of the same buffer for 100 h resulting in an inactive apoenzyme.

2.2. *Neutron activation analysis of the enzymes*

170–280 μg of both holoenzyme and apoenzyme were freeze dried, sealed in silica vials and irradiated in a thermal neutron flux of 6×10^{13} neutrons cm^{-2} sec^{-1} in the Avogadro reactor, Camen, Pisa.

Standards (NBS reference materials) as well as blanks were processed in a similar way and irradiated simultaneously with the samples. The neutron

activated samples were mineralized by wet-ashing digestion, ^{69m}Zn and ^{65}Zn separated from interfering radionuclides by ion-exchange chromatography and counted by computer coupled γ-ray spectrometry using a Ge(Li) detector [11].

2.3. *The binding of Zn to holoenzyme and apoenzyme*

Terminal transferase was incubated with 1 mM Zn^{2+} ions at 25°C for 1 h. The excess Zn^{2+} was removed from the solution by dialysis and the enzyme-bound Zn was determined by neutron activation analysis.

The native enzyme was chromatographed on a small Sephadex G-25 column (0.7 X 1.0 cm). Since preliminary experiments showed that the enzyme-bound Zn^{2+} ions exchange with exogenous Zn^{2+} ions in the buffers and that the Sephadex resin contains Zn [12] the column was previously equilibrated with 0.1 mM ^{65}Zn-labelled Zn^{2+} ions in cacodylate buffer, pH = 6.8 and 0.1 mM mercaptoethanol. The fractions containing the transferase activity were pooled, freeze-dried and the enzyme-bound Zn was determined by neutron activation analysis.

The apoenzyme was incubated with 1 mM Zn^{2+} ions plus 100 μCi carrier-free $^{65}Zn^{2+}$ at 25°C for 1 h. The excess $^{65}Zn^{2+}$ was removed by dialysis and the amount of Zn incorporated into the apoenzyme was estimated by direct measurement of ^{65}Zn radioactivity.

The apoenzyme was chromatographed on a Sephadex G-25 column in a similar way described above for the native enzyme. The fractions from the column containing the transferase activity were pooled and the Zn bound to the reconstituted enzyme was determined by direct measurement of ^{65}Zn radioactivity.

3. Results and discussion

The preparations of terminal transferase with specific activities of 13 400 and 16 100 units had a Zn content of 0.78 and 0.91 g atoms of Zn/mol of mol. wt 33 000 [13] respectively. All other elements detected, such as Au, Cd, Co, Cs, Cu, Fe, Mg, Mn, Ni, P and Sb, were present in amount less than 0.08 g atoms of each metal/33 000. Figure 1 illustrates a

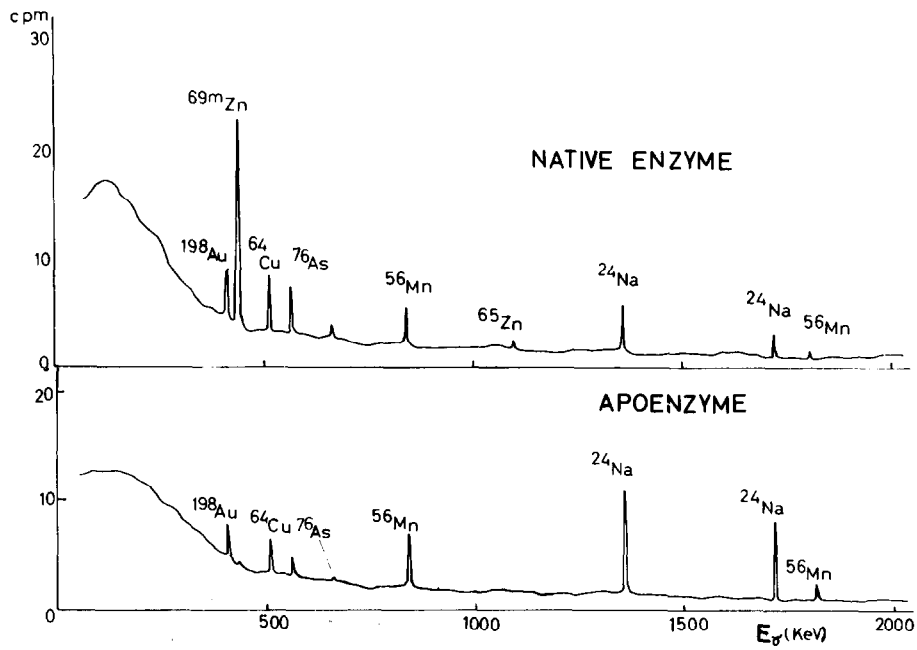

Fig.1. The γ-ray spectrum of neutron activated calf thymus deoxynucleotidyl transferase (A) and apoenzyme (B). The γ-ray spectrum was obtained by high resolution coupled γ-ray spectrometry by means of a 40 cm^3 Ge(Li) detector.

typical γ-ray spectrum of neutron activated terminal transferase [14].

The removal of the Zn from the enzyme resulted in a Zn free apoenzyme (fig.1) retaining less than 8% of the original activity (table 1).

The bound Zn of the native enzyme increases to about 2 g atoms of Zn/33 000 with an apparent increase of its catalytic activity when the enzyme was incubated with an excess of Zn^{2+} ions. The inactive Zn-free apoenzyme was able to incorporate about 1.3–1.45 g atom of Zn/33 000 when incubated with excess $^{65}Zn^{2+}$ ions.

Chromatographic experiments with Sephadex G-25 on both the native enzyme and the apoenzyme after equilibration of the column with 0.1 mM $^{65}Zn^{2+}$ ions showed that Zn was tightly bound to the enzyme since a peak of ^{65}Zn radioactivity was eluted after the void volume associated with a protein peak containing the transferase activity.

Evidently the Zn is firmly bound to the protein and is directly related to one enzymatic activity, strongly indicating that terminal deoxynucleotidyl transferase can be considered as a Zn enzyme in which Zn has a functional role in the catalytic step.

However, no definite conclusions about the stoichiometry of Zn at the maximal enzymatic activity can be drawn from our present results. Although the preparations of the enzyme contained about 1 g atom of Zn/33 000 chromatographic and radiotracing experiments on both the native and the apoenzyme show that the protein is able to bind further Zn up to about 2 g atoms of Zn/33 000 with an apparent increase of transferase activity.

These discrepances can be explained by the great experimental difficulties encountered during this work since in our experimental conditions the enzyme tends to inactivate after dilution or prolonged dialysis with a partial loss of Zn and a decrease in its activity.

References

[1] Yoneda, N. and Bollum, F. J. (1964) J. Biol. Chem. 240, 3385–3391.
[2] Lieberman, I., Abrams, R., Hunt, N. and Ove, P. (1963) J. Biol. Chem. 238, 3955–3962.
[3] Lieberman, I., Abrams, N. H. and Ove, P. (1963) J. Biol. Chem. 238, 2141–2149.
[4] Bollum, F. J. (1966) in: Procedures in Nucleic Acid Research (Cantoni, G. and Davis, D., eds) pp. 296–592 Harper and Row, Inc. New York.
[5] Chang, L. N. S. and Bollum, F. J. (1970) Proc. Natl. Acad. Sci. USA 65, 1041–1048.
[6] Slater, J. P., Mildran, A. S. and Loeb, L. W. (1971) Biochim. Biophys. Acta 44, 37–43.
[7] Loeb, L. A. (1969) J. Biol. Chem. 244, 1672–1681.
[8] Coleman, J. E. (1974) Biochim. Biophys. Acta 60 641–648.
[9] Auld, D. S., Kawaguchi, H., Livingstone, D. M. and Valle, B. L. (1974) Biochem. Biophys. Res. Commun. 62, 296–302.
[10] Sabbioni, E., Girardi, F. and Marafante, E. (1974) Rep. Eur. 5333.
[11] Sabbioni, E. and Girardi, F. (1976) in preparation.
[12] Sabbioni, E. (1976) unpublished observations. We found that Sephadex G-25 contained about 5 μg of Zn/g dry resin, as determined by neutron activation analysis.
[13] Chang, L. M. S. and Bollum, F. J. (1971) J. Biol. Chem. 246, 909–916.
[14] Guzzi, G., Pietra, R. and Sabbioni, E. (1974) Rep. Eur. 5282.