Purification and properties of suppressor seryl-tRNA:ATP phosphotransferase from bovine liver

Takaharu Mizutani and Atsushi Hashimoto

Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467, Japan

Received 6 February 1984; revised version received 7 March 1984

Seryl-tRNA$^{Ser}_{CmCA}$:ATP phosphotransferase was purified 1200-fold from bovine liver by ultracentrifugation at 150000 × *g*, chromatography on DEAE–cellulose, fractional precipitation with ammonium sulfate, chromatography on hydroxyapatite, gel filtration on Sephacryl S-300 and affinity chromatography on Blue Sepharose. Molecular mass was estimated as 135–145 kDa. The K_m values for ATP and ser-tRNA$^{Ser}_{CmCA}$ were 2 mM and 21 nM, respectively. This enzyme did not react with ser-tRNA$^{Ser}_{IGA}$, tyr-tRNA or thr-tRNA.

Bovine liver suppressor serine tRNA *Phosphoseryl-tRNA* *Bovine liver phosphotransferase* *Serine tRNA* *Phosphotransferase purification* *Suppressor seryl-tRNA:ATP phosphotransferase*

1. INTRODUCTION

It has been reported that one tRNA^{Ser} accepts phosphate [1] and one corresponds to codon UGA as suppressor tRNA [2]. These two tRNAs^{Ser} were identical [3] and the primary structure of this tRNA from bovine liver [4] and the tRNA gene structure of chicken were determined [5]. The tRNA plays a suppressor role in in vitro globin synthesis [3], but it is unclear whether phosphoserine on the tRNA is incorporated in a polypeptide chain of protein synthesis.

Many biochemists have become interested in phosphorylation of tyrosine residues [7] or serine residues [8] on proteins, relating to biological functions such as a second messenger and tumor promotion. Meanwhile, the presence of seryl-tRNA:ATP phosphotransferase to produce phosphoseryl-tRNA has been reported [6] but the enzyme has not been purified. We report here the purification and some properties of seryl-tRNA$^{Ser}_{CmCA}$:ATP phosphotransferase (ser-tRNA PTase) in bovine liver. The enzyme was purified from bovine liver 1200-fold by ultracentrifugation at 150000 × *g*, chromatography on DEAE–cellulose, fractional precipitation with ammonium sulfate, chromatography on hydroxyapatite, gel filtration on Sephacryl S-300 and affinity chromatography on Blue Sepharose, as one band on disc gels. The enzyme showed the following properties: molecular mass was estimated as 135–145 kDa, the K_m values for ATP and ser-tRNA$^{Ser}_{CmCA}$ were 2 mM and 21 nM, respectively. This ser-tRNA PTase did not react with ser-tRNA$^{Ser}_{IGA}$ and periodate-oxidized tRNA$^{Ser}_{IGA}$ did not inhibit the phosphotransfer reaction to ser-tRNA$^{Ser}_{CmCA}$. This enzyme did not react with tyr-tRNA or thr-tRNA.

2. EXPERIMENTAL

Bovine liver tRNA was prepared as in [9]. Minor tRNA^{Ser}, which accepted serine and phosphate, was separated as suppressor tRNA$^{Ser}_{CmCA}$ from major tRNA$^{Ser}_{IGA}$ by chromatography on BD–cellulose [6] and used as the substrate of ser-tRNA PTase. The specific content of tRNA$^{Ser}_{CmCA}$ in the fraction was 57 pmol/A_{260} unit from the amount of serine saturated on tRNA. [γ-^{32}P]ATP was prepared as in [10] and [^{14}C]serine was a product (25 Ci/mol) of the Radiochemical Centre (Amersham). To determine the K_m values for ser-tRNA$^{Ser}_{CmCA}$ by ser-tRNA PTase, [^{14}C]seryl-tRNA was prepared by aminoacylation of tRNA$^{Ser}_{CmCA}$ with seryl-tRNA synthetase as in [11]. The oxidized tRNA$^{Ser}_{IGA}$ with

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

periodate was prepared from the major $tRNA^{Ser}$ fraction on BD–cellulose as in [12].

To measure the purification of ser-tRNA PTase, the reaction mixture (100 μl), composed of 0.2 M Tris–HCl (pH 7.6), 0.02 M KCl, 0.02 M $MgCl_2$, 0.8 μM $tRNA^{Ser}_{CmCA}$, 0.2 mM serine and 5 mM [γ-^{32}P]ATP (1000 cpm/nmol), was mixed with 10 μl seryl-tRNA synthetase (0.5 mg/ml) and incubated for 10 min at 37°C. Then the reaction was started by mixing with 10 μl of each ser-tRNA PTase preparation (0.1–10 mg/ml). After incubation for 2–5 min at 37°C, the reaction mixture was spotted on filter paper which was then immersed in 10% trichloroacetic acid to stop the reaction and the radioactivity was counted [11]. The assay was duplicated. Electrophoresis on gels was done as in [13,14]. Protein concentration was determined by measurement of the absorbance at 280 nm or as in [15]. All purification procedures were performed in a cold room at 4°C. The buffer used for chromatography except for that on hydroxyapatite was 10 mM Tris–HCl (pH 7.6), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol and 5% glycerol (buffer A). The buffer used for chromatography on hydroxyapatite was 10 mM Tris–HCl in buffer A (buffer B).

3. RESULTS AND DISCUSSION

Bovine liver (500 g) was minced and ground with quartz sands in 2.5 l of 0.25 M sucrose, 10 mM Tris–HCl (pH 7). The solution was further minced for 30 min at 4°C, centrifuged at 150000 × g for 30 min and the supernatant containing 45 g protein was applied on DEAE–cellulose, which was previously equilibrated with buffer A and eluted with a linear gradient of KCl (fig.1A). Ser-tRNA PTase was eluted at 0.18 M KCl and the active fraction of tubes 131–147 (fig.1A) was mixed with saturated ammonium sulfate solution to bring to 0.3 saturation of ammonium sulfate. The solution was centrifuged and then saturated ammonium sulfate solution was added to the supernatant to achieve 0.5 saturation. The precipitate was collected and dissolved in buffer A. A part (430 mg) of the solution was chromatographed on Sephacryl S-300 (fig.1B). The activity was eluted at K_d 0.20. The active fraction of tubes 36–41 (fig.1B) was dialyzed against buffer B for 4 h and then chromatographed on hydroxyapatite with a linear gradient (total 400 ml) from buffer B to 0.4 M potassium phosphate in buffer B (fig.1C). The active fraction eluted at 0.22 M phosphate (fig.1C) was diluted with buffer A to 3 vols and chromatographed on Blue Sepharose with a linear gradient of KCl (total volume 200 ml). The activity was eluted at 0.7 M KCl (fig.1D). The results of purification are summarized in table 1. Ser-tRNA PTase was purified

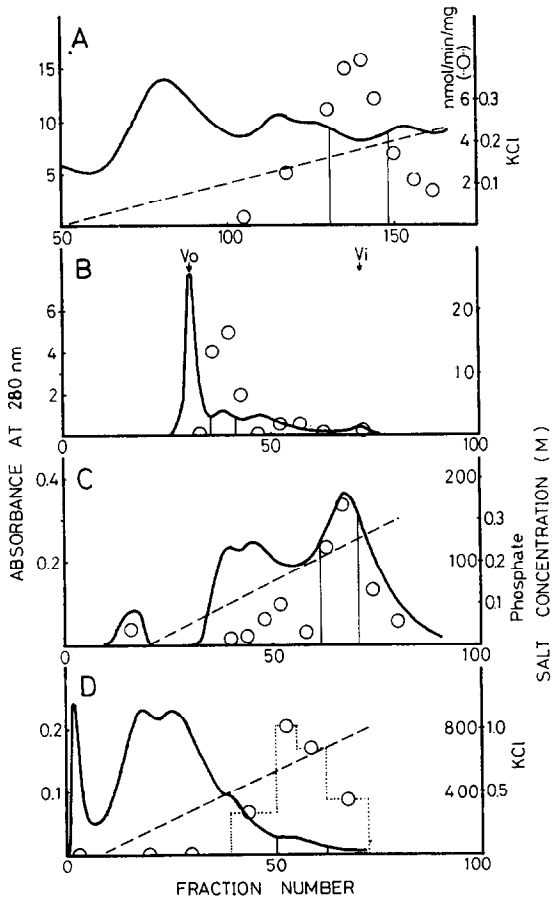

Fig.1. Elution pattern of ser-tRNA phosphotransferase. (A) A supernatant (45 g protein) at 150000 × g was chromatographed on a DEAE–cellulose column (6 cm internal diameter × 40 cm height) with a fraction volume of 20 ml; (B) a sample (430 mg) was chromatographed on Sephacryl S-300 (3.7 × 70 cm) with a fraction volume of 10 ml (V_o, void volume; V_i, inner volume); (C) a sample (100 mg) was chromatographed on a hydroxyapatite column (2 × 35 cm) with a fraction volume of 4.5 ml; (D) a sample (30 mg) was chromatographed on a Blue Sepharose column (1.2 × 8.5 cm) with a fraction volume of 1.5 ml. Salts used for gradient elution were KCl for A and D, and potassium phosphate for C. Other details are described in the text.

Table 1

Summary of purification of bovine liver seryl-tRNA:ATP phosphotransferase

Step	Total protein (mg)	Total activity (nmol/min)	Specific activity (nmol/min per mg)	Yield (%)	Relative purification
Supernatant	45 000	2.97×10^4	0.66	100	1
DEAE–cellulose	3 100	2.33×10^4	7.5	78	11
Fractional precipitation with ammonium sulfate (0.3–0.5 saturation)	690	7.59×10^3	11.0	26	17
Sephacryl S-300	114	2.90×10^3	25	10	38
Hydroxyapatite	20	2.70×10^3	130	9	190
Blue Sepharose	1.3	0.98×10^3	790	3.3	1200

1200-fold with a recovery of 3.3%. This enzyme was dialyzed against 50% glycerol–10 mM Tris–HCl, 10 mM β-mercaptoethanol and 2 mM EDTA for preservation and stored at −20°C. The enzyme activity in 50% glycerol at −20°C was stable for 1 year or longer.

The final preparation in fig.1D showed one band on a disc gel by the method of [13] but two bands on an SDS disc gel by the method of [14]. Therefore, it was concluded that the enzyme was composed of subunits or was a partially purified preparation. The molecular mass of this ser-tRNA PTase was estimated from the K_d value (0.20) on Sephacryl S-300 to be 145 kDa using the calibration curve of standard proteins. The main band on the SDS disc gel moved to 135 kDa near the band of the bovine serum albumin dimer. From these results, the molecular mass of ser-tRNA PTase was 135–145 kDa.

Fig.2 shows Lineweaver-Burk plots of the phosphotransfer reaction catalyzed by ser-tRNA PTase for ATP and ser-tRNA. The K_m value for ATP was 2 mM and similar to that (0.5 mM) of ser-tRNA synthetase. The K_m value for ser-tRNA was 21 nM and lower than that of ser-tRNA synthetase for $tRNA^{Ser}$ (1.5 μM). Ser-tRNA PTase has about 100-fold higher affinity to ser-$tRNA^{Ser}_{CmCA}$ compared to ser-tRNA synthetase. This property corresponds well to the biological function of the PTase, because the concentration of suppressor serine tRNA is low and is estimated to be about 50 nM in cytoplasm. Therefore it is reasonable to suppose that ser-tRNA PTase has high affinity to suppressor tRNA. This result also supports the possibility that phosphoseryl-$tRNA^{Ser}_{CmCA}$ plays an important biological role in mammals. One of the possible roles of phosphoseryl-tRNA is in protein synthesis and another in the reverse metabolic pathway from serine to 3-phosphohydroxypyruvate.

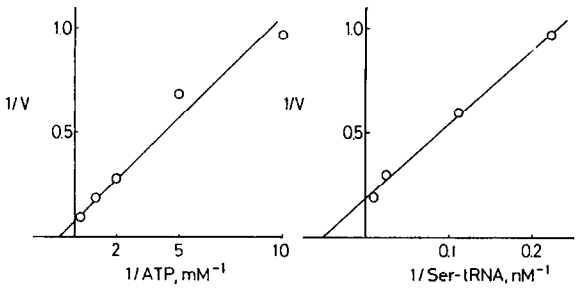

Fig.2. Lineweaver-Burk plots of the phosphotransfer reaction catalyzed by ser-tRNA:ATP phosphotransferase with ATP (left) and suppressor ser-tRNA (right).

Purified ser-tRNA PTase did not react with major ser-$tRNA^{Ser}_{IGA}$, therefore this enzyme must recognize some regions on the tRNA structure. The sequence of rat liver $tRNA^{Ser}_{IGA}$ [16] differed from that of bovine $tRNA^{Ser}_{CmCA}$ [4] at many regions. These regions should be contained in the recognition sites on tRNA by ser-tRNA PTase. This was supported by the experimental result that periodate-oxidized $tRNA^{Ser}_{IGA}$ did not inhibit the phosphotransfer reaction from ATP to ser-$tRNA_{CmCA}$ by ser-tRNA PTase. In addition, this enzyme did not produce phosphotyrosyl-tRNA or phosphothreonyl-tRNA from tyr-tRNA and ATP or thr-tRNA and ATP.

REFERENCES

[1] Mäenpää, P.H. and Bernfield, M.R. (1970) Proc. Natl. Acad. Sci. USA 67, 688–695.
[2] Hatfield, D. and Portugal, F.H. (1970) Proc. Natl. Acad. Sci. USA 67, 1200–1206.
[3] Hatfield, D., Diamond, A. and Dudock, B. (1982) Proc. Natl. Acad. Sci. USA 79, 6215–6219.
[4] Diamond, A., Dudock, B. and Hatfield, D. (1981) Cell 25, 497–506.
[5] Hatfield, D.L., Dudock, B.S. and Eder, F.C. (1983) Proc. Natl. Acad. Sci. USA 80, 4940–4944.
[6] Sharp, S.J. and Stewart, T.S. (1977) Nucleic Acids Res. 4, 2123–2136.
[7] Witte, N.O., Dasgupta, A. and Baltimore, D. (1980) Nature 283, 826–831.
[8] Imaoka, T., Lynham, J.A. and Haslam, R.J. (1983) J. Biol. Chem. 258, 11404–11414.
[9] Narihara, T., Fujita, Y. and Mizutani, T. (1982) J. Chromatogr. 236, 513–518.
[10] Glynn, I.M. and Chappell, J.B. (1964) Biochem. J. 90, 147–149.
[11] Mizutani, T. (1983) J. Biochem. 94, 163–169.
[12] Furuichi, Y. and Miura, K. (1972) J. Mol. Biol. 64, 619–632.
[13] Davis, B.J. (1964) Ann. NY Acad. Sci. 121, 404–427.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Rogg, H., Mueller, P. and Staehelin, M. (1975) Eur. J. Biochem. 53, 115–127.