Identification of three protein kinases which phosphorylate threonyl-tRNA synthetase from rat liver

Ann Marie Pendergast and Jolinda A. Traugh*

Department of Biochemistry, University of California, Riverside, CA 92521, USA

Received 12 August 1986

Threonyl-tRNA synthetase is phosphorylated in Chinese hamster ovary cells labeled with $^{32}P_i$ [(1984) J. Biol. Chem. 259, 11160–11161]. Phosphorylation of the purified synthetase from rat liver has been examined with five different protein kinases. Three of the enzymes phosphorylate the synthetase, protease activated kinase I, the cAMP-dependent protein kinase, and the Ca^{2+}, phospholipid-dependent protein kinase. Phosphorylation occurs exclusively on seryl residues. Two-dimensional phosphopeptide maps of tryptic digests of the phosphorylated synthetase are distinct with each protein kinase. These data suggest that multiple phosphorylation of the synthetase may occur in vivo.

Threonine *tRNA synthetase* *Protein kinase* *Phosphorylation*

1. INTRODUCTION

Interconversion of enzymes between their phosphorylated and dephosphorylated forms is an extremely versatile mechanism for controlling metabolic processes, including protein synthesis [1]. Recently, Gerken and Arfin [2] demonstrated that threonyl-tRNA synthetase is phosphorylated on serine in mutant Chinese hamster ovary cells following incubation of the cells with $^{32}P_i$. The protein kinase(s) responsible for this phosphorylation was not identified. Mathews and Bernstein [3] found that histidyl-tRNA synthetase was also phosphorylated in wild-type HeLa cells and Gerken et al. [4] substantiated this observation in Chinese hamster ovary cells. Traugh and Pendergast [5] found that five of the eight amino acyl-tRNA synthetases in the high molecular mass complex were phosphorylated following labeling of reticulocytes with $^{32}P_i$; these included the glutamyl-, methionyl-, glutaminyl-, lysyl- and aspartyl-tRNA synthetases. Thus, seven out of nine synthetases examined have been shown to be phosphorylated in vivo.

Previously, we showed four amino acyl-tRNA synthetases in the high molecular mass complex from reticulocytes were phosphorylated in vitro by casein kinase I and phosphorylation was accompanied by a decreased affinity for tRNA and inhibition of amino acylation [6]. In this report, highly purified threonyl-tRNA synthetase from rat liver, an α_2 dimer with a subunit molecular mass of 85 kDa [7], was examined as substrate for five different protein kinases; three were shown to phosphorylate the synthetase.

2. EXPERIMENTAL

2.1. *Materials*

Casein kinase I, casein kinase II and protease activated kinase (PAK) I were highly purified from rabbit reticulocytes as described [8–10]. The catalytic subunit of the cAMP-dependent protein kinase (cA kinase) from bovine muscle was a gift of Dr Edwin G. Krebs, University of Washington, Seattle, WA. The Ca^{2+}, phospholipid-dependent

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

protein kinase (C kinase), was purified from rabbit liver by chromatography on DEAE-cellulose and protamine-agarose [11]. C kinase from beef heart was a generous gift of Dr Donal A. Walsh, University of California, Davis, CA. Threonyl-tRNA synthetase, highly purified from rat liver [7] was generously provided by Dr Murray P. Deutscher, University of Connecticut Health Center, Farmington, CT.

2.2. *Phosphorylation of threonyl-tRNA synthetase*

Threonyl-tRNA synthetase was incubated with 100 units of each of the following protein kinases: casein kinase I, casein kinase II, cA kinase, PAK I and C kinase. One unit of enzyme activity was defined as the amount of enzyme which catalyzed the incorporation of one pmol of inorganic phosphate from [γ-^{32}P]ATP into standard substrate per min at 30°C. The substrate was dephosphorylated casein for casein kinase I and II, mixed histone for C kinase and PAK I, and histone 1 for C kinase. The first four protein kinases were examined in reaction mixtures of 0.07 ml containing 50 mM Tris-HCl, pH 7.4, at 30°C, 30 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 0.2 mM [γ-^{32}P]ATP (spec. act. 832 cpm/pmol), threonyl-tRNA synthetase (0.75 μg) and protein kinase. Prior to adding PAK I to the phosphorylation reaction, the enzyme was activated by limited digestion with trypsin as described by Tuazon et al. [12]. Incubation was for 30 min at 30°C and phosphorylation was terminated by the addition of gel electrophoresis sample buffer. The sample was analyzed by gel electrophoresis on 7.5% polyacrylamide gels followed by autoradiography as described [13]. Phosphorylation with C kinase was carried out as described above except that $MgCl_2$ was 5 mM, 0.3 mM EGTA was added, and the following compounds were present as indicated, 0.5 mM $CaCl_2$, 10 μg/ml phosphatidylserine, 10 μg/ml phosphatidylinositol and 0.8 μg/ml diolein.

2.3. *Phosphoamino acid analysis and phosphopeptide mapping*

Threonyl-tRNA synthetase (0.75 μg) was phosphorylated with cA kinase (175 units), PAK I (58 units) or C kinase (30 units). Following electrophoresis on polyacrylamide gels, the phosphorylated synthetase was excised and the phosphoamino acids were analyzed as described [6].

For two-dimensional phosphopeptide mapping, the synthetase was digested with trypsin [6]. The digest was lyophilized, resuspended in 0.02 ml of water and applied to a thin-layer cellulose sheet. Electrophoresis was carried out at 600 V for 2 h in the first dimension in pyridine:acetic acid:water (1:10:89), pH 3.5. Ascending chromatography was performed in butanol:pyridine:acetic acid:water (60:40:12:48). The labeled phosphopeptides were identified by autoradiography.

3. RESULTS AND DISCUSSION

Threonyl-tRNA synthetase was examined as a potential substrate for five purified protein kinases since the synthetase has been shown to be phosphorylated in Chinese hamster ovary cells incubated with $^{32}P_i$ [2]. As indicated in fig.1, cA kinase and PAK I effectively phosphorylated threonyl-tRNA synthetase, while casein kinase I and casein kinase II were ineffective. Threonyl-tRNA synthetase was also phosphorylated by C kinase and phosphorylation was shown to be dependent on Ca^{2+} and phospholipids (fig.2).

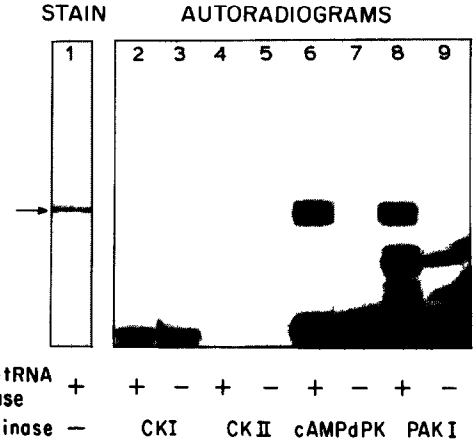

Fig.1. Analysis of threonyl-tRNA synthetase with casein kinase I, casein kinase II, cA kinase and PAK I. Threonyl-tRNA synthetase was incubated with casein kinase I (CK I), casein kinase II (CK II), cA kinase (cAMPdPK), and PAK I as described in section 2 and analyzed by polyacrylamide gel electrophoresis and autoradiography. Lane 1, stained protein pattern of purified threonyl-tRNA synthetase; lanes 2–9, autoradiogram.

Phosphoamino acid analysis of threonyl-tRNA synthetase phosphorylated by cA kinase, PAK I and C kinase, showed that all three protein kinases modified exclusively seryl residues (fig.3). This coincides with the data of Gerken and Arfin [2] showing that phosphorylation of threonyl-tRNA synthetase in Chinese hamster ovary cells occurred on serine.

To determine whether phosphorylation of threonyl-tRNA synthetase by the three protein kinases was on the same or different sites, the radiolabeled synthetase was analyzed by two-dimensional phosphopeptide mapping. As shown in fig.4, each protein kinase gave a distinctive phosphopeptide pattern. The two-dimensional phosphopeptide map of threonyl-tRNA synthetase phosphorylated by PAK I contained four major and one minor phosphopeptide, while those obtained following phosphorylation by the cA kinase and C kinase contained two phosphopeptides each.

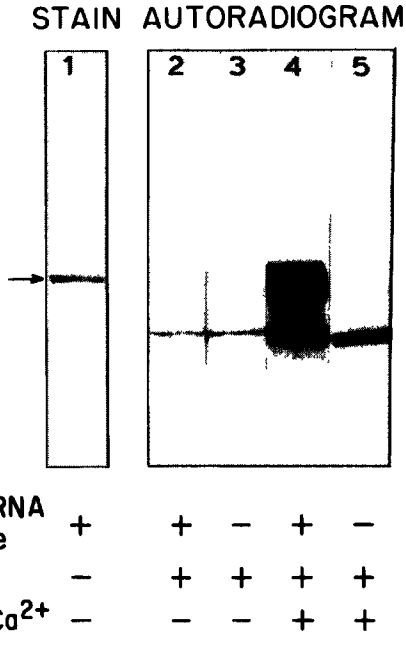

Fig.2. Phosphorylation of threonyl-tRNA synthetase by C kinase. Threonyl-tRNA synthetase was phosphorylated with C kinase under the conditions indicated and analyzed by polyacrylamide gel electrophoresis followed by autoradiography. Lane 1, stained protein pattern of threonyl-tRNA synthetase; lanes 2–5, autoradiogram.

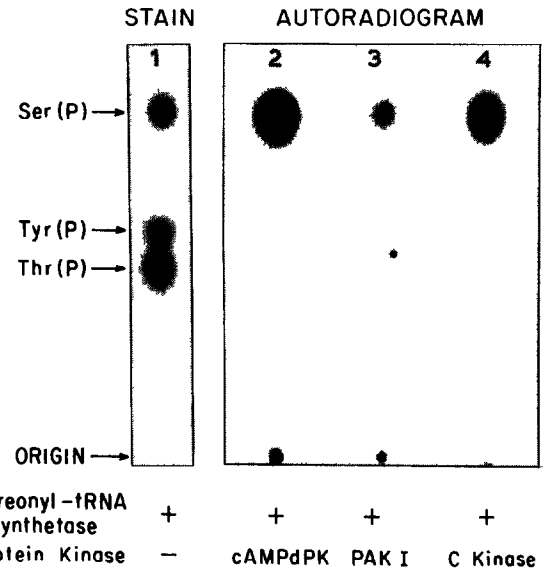

Fig.3. Phosphoamino acid analysis of threonyl-tRNA synthetase phosphorylated by cA kinase (cAMPdPK), PAK I, and C kinase. Lane 1, phosphoamino acid standards visualized with ninhydrin; lanes 2–4, autoradiogram.

Recently, the amino acid sequence of the yeast threonyl-tRNA synthetase has been deduced from the corresponding nucleotide sequence of the cloned gene [14]. Of the 47 serine residues in the primary sequence, there is one site containing a sequence similar to the consensus sequence recognized by the cA kinase, Arg-Arg-X-Ser [15]. There are at least six serines with a sequence similar to that recognized by PAK I, Lys-X-X-Ser [16]. Turner et al. [17] indicated that the sequence for C kinase was similar to that established for cA kinase, while Nishizuka and co-workers [18] indicated that the majority of substrates for C kinase also contained one or more basic amino acids at the C-terminus of the phosphorylated residue. Using the criteria of Nishizuka and co-workers, there are 13 possible recognition sequences in yeast threonyl-tRNA synthetase. Amino acid analysis of the mammalian threonyl-tRNA synthetase indicates 44 serines are present per 85 kDa subunit [7]. Assuming the mammalian enzyme has a degree of homology with the yeast enzyme, there should be numerous potential sites for the protein kinases.

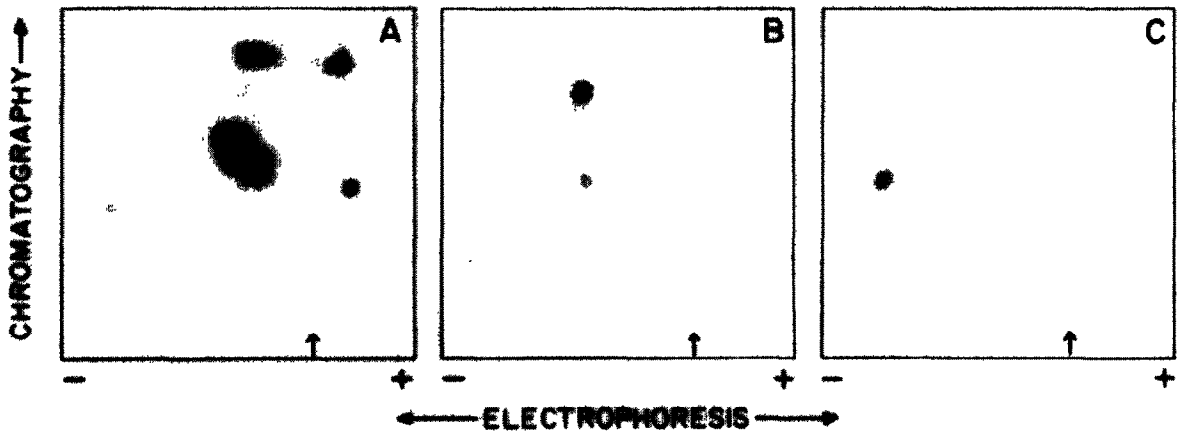

Fig.4. Phosphopeptide maps of threonyl-tRNA synthetase phosphorylated by PAK I, cA kinase and C kinase. Threonyl-tRNA synthetase was phosphorylated with PAK I (A), cA kinase (B), and C kinase (C); tryptic digests of the synthetase were analyzed by phosphopeptide mapping as described in section 2. Autoradiograms of the phosphopeptides are shown. The origin is indicated by an arrow.

The fact that the threonyl-tRNA synthetase is phosphorylated by at least three different protein kinases is consistent with observations obtained with numerous other phosphoproteins. In almost every instance, a protein phosphorylated at one or more sites by a single protein kinase is also phosphorylated at other sites by additional protein kinases.

ACKNOWLEDGEMENTS

We wish to thank Dr Murray P. Deutscher for the generous gift of highly purified threonyl-tRNA synthetase, Dr E.G. Krebs for generously providing the catalytic subunit of the cA kinase and Dr Donal A. Walsh for C kinase. This research was funded by US Public Health Service grant GM 21424.

REFERENCES

[1] Traugh, J.A. (1981) in: Biochemical Actions of Hormones, vol.8, pp.167–208, Academic Press, New York.
[2] Gerken, S.C. and Arfin, S.M. (1984) J. Biol. Chem. 259, 11160–11161.
[3] Mathews, M.B. and Bernstein, R.M. (1983) Nature 304, 177–179.
[4] Gerken, S.C., Andrulis, I.L. and Arfin, S.M. (1986) Biochim. Biophys. Acta 869, 215–217.
[5] Traugh, J.A. and Pendergast, A.M. (1986) Prog. Nucleic Acids Res. Mol. Biol. 33, 195–230.
[6] Pendergast, A.M. and Traugh, J.A. (1985) J. Biol. Chem. 260, 11769–11774.
[7] Dignam, J.D., Rhodes, D.G. and Deutscher, M.P. (1980) Biochemistry 19, 4978–4984.
[8] Hathaway, G.M., Tuazon, P.T. and Traugh, J.A. (1983) Methods Enzymol. 99, 308–317.
[9] Hathaway, G.M. and Traugh, J.A. (1979) J. Biol. Chem. 254, 762–768.
[10] Tahara, S.M. and Traugh, J.A. (1981) J. Biol. Chem. 256, 11558–11564.
[11] Pendergast, A.M. (1986) PhD Dissertation, University of California, Riverside.
[12] Tuazon, P.T., Merrick, W.C. and Traugh, J.A. (1980) J. Biol. Chem. 255, 10954–10958.
[13] Hathaway, G.M., Lundak, T.S., Tahara, S.M. and Traugh, J.A. (1979) Methods Enzymol. 60, 495–511.
[14] Pape, L.K. and Tzagoloff, A. (1985) Nucleic Acids Res. 13, 6171–6183.
[15] Krebs, E.G. and Beavo, J.A. (1979) Annu. Rev. Biochem. 48, 923–959.
[16] Leis, J., Johnson, S., Collins, L.S. and Traugh, J.A. (1984) J. Biol. Chem. 259, 7726–7732.
[17] Turner, R.S., Kemp, B.E., Su, H.-D. and Kuo, J.F. (1985) J. Biol. Chem. 260, 11503–11507.
[18] Kishimoto, A., Nishiyama, K., Nakanishi, H., Uratsuyi, Y., Nomura, H., Takeyama, Y. and Nishizuka, Y. (1985) J. Biol. Chem. 260, 12492–12499.