Volume 309, number 3, 337–339 FEBS 11528 September 1992

Carbohydrates in mammalian tryptophanyl-tRNA synthetase

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Received 15 July 1992

Homogeneous preparations of bovine tryptophanyl-tRNA synthetase (EC 6.1.1.2) contain monosaccharides (mannose, fucose, galactose, *N*-acetylglucosamine) as revealed by liquid chromatography. Their content comprises 2.5–3.0% (w/w) of the enzyme composed of two subunits (60 kDa × 2). The same set of sugars was detected in elastase and CNBr-generated fragments (with molecular masses of approx. 40 kDa and 30 kDa, respectively). It is concluded that bovine tryptophanyl-tRNA synthetase, in addition to being a metallo- and phosphoprotein, is also a glycoprotein.

Tryptophanyl-tRNA synthetase; Beef; Sugar; Glycoprotein; Mammal; Aminoacyl-tRNA synthetase

1. INTRODUCTION

At present, the main approach to the determination of the primary structure of proteins is undoubtedly molecular cloning of their structural genes (cDNAs) and deciphering their nucleotide sequences. Although this approach is very efficient and widespread it is unable to reveal the presence of non-protein components of the natural proteins like metals, carbohydrates, lipids, phosphates, methyl groups etc. However, for enzymes and other proteins these components very often play a key role in their functioning, compartmentation in a living cell, traffic etc. The only way to eliminate this drawback of gene engineering technology is to analyze highly purified protein preparations directly by modern analytical techniques.

The primary structures of mammalian aminoacyl-tRNA synthetases were determined only by gene-engineering approach (see [1]) and post translational modification of these proteins remains to be studied. It is known that some mammalian aminoacyl-tRNA synthetases contain carbohydrates [2,3], although the function of these residues was not established.

It is shown in this work that bovine tryptophanyl-tRNA synthetase (WRS) is a glycoprotein containing 2.5–3% by weight of carbohydrates. To make this conclusion reliable it is important to analyze highly purified enzyme preparations. To meet this requirement the WRS preparation obtained as described earlier [4] was subjected to the additional procedures: separation on Toyo-Pearl HW-65 by hydrogen bonding chromatography and/or size exclusion HPLC on a TSK-3000 (Altex) column. The homogeneity of the preparations obtained by this way was checked by SDS- PAGE and immuno electrophoresis.

Native protein, its proteolytic and CNBr-fragments were purified and analyzed for the presence of monosaccharides by liquid chromatography; mannose, fucose, galactosamine and *N*-acetylglucosamine were revealed. Taking into account these data one may classify the WRS as belonging to the group of *N*-glycosylated proteins; this protein modification appears co-translationally in eukaryotic cells [5].

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Abbreviations: AmS, ammonium sulfate; DTT, dithiotreitol; 2-ME, 2-mercaptoethanol; WRS, bovine tryptophanyl-tRNA synthetase; TFA, trifluoroacetic acid.

2. MATERIALS AND METHODS

WRS was purified as described earlier [4], but the last step of purification (Sephadex G-150 chromatography) was omitted. Instead the DEAE fraction was precipitated by 60% saturated AmS at 4°C, the suspension was transferred to a Toyo-Pearl HW-65 (Toyo- Soda) column (1.5 × 13.0 cm) equilibrated with 60% AmS, containing 0.1 M Tris-HCl, pH 7.5. The gradient elution with AmS (60%–10% saturation) was applied using the same Tris buffer. The gradient volume was 456 ml, and the flow rate 30 ml/h. The main protein fraction eluted between 215 and 240 ml was precipitated with 60% AmS, the sediment was dissolved in 20 mM Tris-HCl, pH 7.5 with 0.5 mM L-tryptophan and 0.5 mM DTT to stabilize the enzyme [4]. The solution was subjected to acid precipitation as described [4] to remove traces of leucine aminopeptidase [4]. The residue was dissolved in 3 ml of the aforementioned buffer, containing 60% saturated AmS and applied to the Toyo-Pearl HW-65 column (1.5 × 13.0 cm) and the chromatography was repeated as described above; elution volume 285 ml at 70 ml/h.

The enzyme preparations were analyzed by SDS-PAGE [6]. The gels were stained with Coomassie brilliant blue G-250 [6] and with $AgNO_3$ [7].

The 40-kDa fragment of WRS was isolated from elastase hydrolysate (WRS/elastase, 100:1 w/w, 30 min, 30°C) according to [8] and purified as described above for native WRS. Isolation and purification of the 30-kDa fragment after CNBr cleavage will be described elsewhere (Egorov et al., unpublished data).

For determination of the natural monosaccharides 2 nmol of WRS or 4 nmol of its fragments were hydrolysed with 3 M TFA at 100°C for 6 h. For determination of amino sugars, hydrolysis was performed with 4 N HCl under the same conditions. Neutral sugars were chromatographed on Biotronik LC-2000 (DA-X8 column) and amino sugars on Biotronik LC-4000 (Aminex A-5 column) as described [9].

3. RESULTS AND DISCUSSION

The WRS preparations purified according to the previously developed method [4] were 90–95% pure which is not sufficient for analysis of the protein carbohydrate content of the protein since in some cases the sugar moieties may comprise only 1% of the total protein mass [5]. This is why we decided to improve the purification scheme and introduced two additional steps: hydrophobic chromatography in a reversed gradient of AmS density (Fig. 1) and size-exclusion HPLC on TSK-3000 (Altex, 7.5 × 600 mm column, not shown). These preparations turned out to be at least 99% homogeneous on SDS-PAGE and therefore were suitable for further analysis.

All WRS preparations contain monosaccharides as evidenced from the data presented in Table I. The sugar content comprises 2.5–3% the 120-kDa mass of the protein (a dimer).

There is no good stoichiometry for galactose and *N*-acetylglucosamine moieties; probably it is caused by micro-heterogeneity of the enzyme preparation revealed by 2D gel electrophoresis [10].

The same monosaccharides were also detected in the product of WRS after limited proteolysis (40 kDa fragment) and in one of the CNBr fragments with molecular mass of about 30 kDa (see Table I). *N*-Acetylgalactosamine typical for *O*-glycosylated proteins [5] was not detected in native WRS or in its fragments.

Taken together the obtained data show that monosaccharides are covalently linked with the polypeptide chain and therefore WRS is in fact glycosylated. Earlier it was demonstrated that WRS contains Zn^{2+} cation [11] and might be phosphorylated in vivo [12], in cell extracts [13] and by pure casein kinase II [10]. Therefore it may be concluded that bovine WRS is a glyco phospho metalloprotein.

Table I

Monosaccharide composition of bovine tryptophanyl-tRNA synthetase and its fragments (nmol CHO/nmol protein or fragment)

Carbohydrate	WRS, monomer 60 kDa	Fragments	
		40 kDa	30 kDa
Mannose	3.1	2.8	3.0
Fucose	0.4	0.2	2.5
Galactose	0.5	0.3	3.2
N-Acetylglucosamine	+	+	+
N-Acetylgalactosamine	–	–	–

The monosaccharide composition of protein and its fragments was determined as described in section 2.

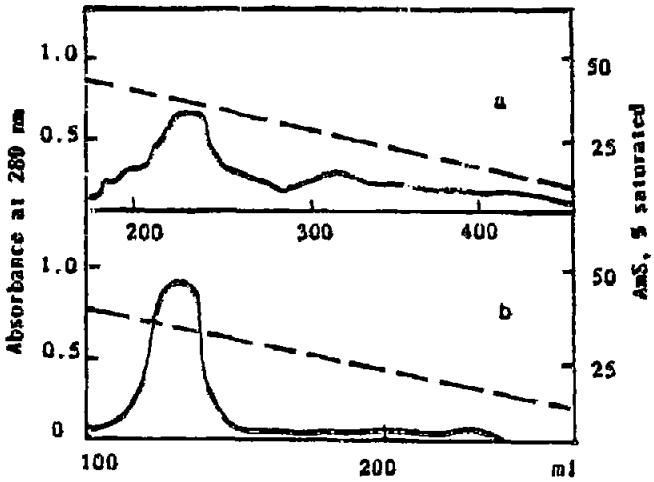

Fig. 1. Toyo-Pearl HW-65 column chromatography of bovine tryptophanyl-tRNA synthetase in linear gradient of 60–10% saturated AmS. (a) DEAE-fraction of the enzyme [4] was applied on the column (1.5 × 13 cm). (b) Rechromatography of the peak fraction (a, 215–240 ml, acid precipitate) on the column (1.5 × 9.0 cm). For details see section 2.

Some known properties of WRS are consistent with the presence of sugars in its structure. Immuno electron microscopy of WRS in animal cell cultures and in pancreas [12,14,15] has shown that this enzyme is associated with the rough endoplasmic reticulum, mitochondria, nuclei, polyribosomes, which is rather typical for glycoproteins [5]. Immunochemically positive WRS fragments are secreted by the pancreatic gland and may be found in pancreatic juice [16]; it is known [5] that secreted proteins are usually glycosylated. WRS has a very strong tendency to aggregate in solution [17] which again is typical for glycoproteins [5]. Glycosylation of WRS may partially explain the apparent non-identity of molecular masses of the WRS polypeptide chain calculated from cDNA structure (53.7 kDa [18] and the 58.0 ± 2 kDa value estimated from SDS-PAGE (see [4]). Finally, in the primary structure of bovine [18] and human [19] WRS two sites were revealed (Asn^{234}–Lys^{235}–Thr^{236}; Asn^{361}–Ser^{362}–Ser^{363} and Asn^{229}–Lys^{230}–Thr^{231}; Asn^{356}–Ser^{357}–Ser^{358}, respectively), that are usually recognized by glycosylation enzymes [5].

REFERENCES

[1] Mirande, M. (1991) Progr. Nucleic Acids Res. 40, 95–142.
[2] Glinski, R.L., Rainey, P.C., Manhinney, T.P. and Hilderman, R.H. (1979) Biochem. Biophys. Res. Commun. 88, 1052–1060.
[3] Dignam, J.D., Rhodes, D.G. and Deutscher, M.P. (1980) Bio-

chemistry 19, 4978–4984.
[4] Kisselev, L.L., Favorova, O.O. and Kovaleva, G.K. (1979) Methods Enzymol. 59, part G, 234–257.
[5] Paulson, J.C. (1989) Trends Biochem. Sci. 14, 272–276.
[6] Laemmli, U.K. (1970) Nature 227, 680–685.
[7] Wrey, W., Boulikas, T., Wray, V.P. and Hancock, R., Analyt. Biochem. 118, 197–203.
[8] Scheinker, V., Beresten, S., Mazo, A., Ambartsumian, N., Rochlin, O., Favorova, O. and Kisselev, L.L. (1978) Eur. J. Biochem. 97, 529–540.
[9] Arbatsky, N.P., Derevitskaya, V.A., Zheltova, A.O., Kochetkov, N.K., Likhosherstov, L.M., Senchenko, S.N. and Yrtov, D.V. (1988) Carbohydrate Res. 178, 165–181.
[10] Elizarov, S.M. and Kovaleva, G.K. (1990) Molekul. Biol. (Moscow) 24, 1616–1623.
[11] Kisselev, L.L., Favorova, O.O., Nurbekov, M.K., Dmitrienko, S.G. and Engelhardt, W.A. (1981) Eur. J. Biochem. 120, 511–517.
[12] Paley, E.L., Baranov, V.N., Alexandrova, N.M. and Kisselev, L.L. (1991) Exp. Cell Res. 195, 66–78.
[13] Zargarova, T.A., Aleksandrova, N.M., Paley, E.L. and Favorova, O.O. (1990) Biochimiya (Moscow) 55, 1328–1338.
[14] Popenko, V.I., Cherny, N.E., Beresten, S.F., Zargarova, T.A. and Favorova, O.O. (1989) Molek. Biol. (Moscow) 23, 1669–1681.
[15] Paley, E.L., Baranov, V.N. and Kisselev, L.L. (1989) Bull. Exp. Biol. Med. (Moscow) 1, 100–103.
[16] Favorova, O.O., Zargarova, T.A., Rukosuyev, V.S., Beresten, S.F. and Kisselev, L.L. (1989) Eur. J. Biochem. 184, 583–588.
[17] Tuzikov, F.V., Tuzikova, N.A., Vavilin, V.I., Zinoviev, V.V., Malygin, E.G., Favorova, O.O., Zargarova, T.A., Sudomoina, M.A. and Kisselev, L.L. (1991) Molekul. Biologia (Moscow) 25, 740–751.
[18] Garret, M., Pajot, B., Trezequet, V., Labouesse, J., Merle, M., Candar, J.-C., Benedetto, J.-P., Sallafranque, M.L., Alterio, J., Gueguen, M., Sarger, C., Labouesse, B. and Bonnet, J. (1991) Biochemistry 30, 7809–7817.
[19] Frolova, L.Yu., Sudomoina, M.A., Grigorieva, A.Yu., Zinovieva, O.L. and Kisselev, L.L. (1991) Gene 109, 291–296.