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Rat Liver Histidyl-tRNA Synthetase. Purification and Inhibition by the Myositis-Specific Anti-Jo-1 Autoantibody

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Myositis is an autoimmune inflammatory muscle disease of unknown etiology. We demonstrate directly that the antigen to the myositis-specific anti-Jo-1 antibody is histidyl-tRNA synthetase. The anti-Jo-1 antibody inhibits human HeLa and rat liver histidyl-tRNA synthetase. Using conventional and immunoaffinity chromatography with immobilized anti-Jo-1 antibody, we have purified rat liver histidyl-tRNA synthetase which has a subunit M_r 64,000 and an estimated native M_r suggesting an α_2 structure. The evidence indicates that the Jo-1 antigen is histidyl-tRNA synthetase, and that some of the histidyl-tRNA synthetase structure are conserved across species.

Many of the autoimmune diseases are associated with autoantibodies directed toward cellular antigens, some of which are nucleoprotein complexes (1). Polymyositis, a potentially devasting inflammatory muscle disease, is associated with the myositis-specific anti-Jo-1 autoantibody which is found in 30% of these patients (2-4). The anti-Jo-1 antibody precipitated a nucleoprotein complex containing $tRNA^{His}$ (5) and inhibited human HeLa His-tRNA synthetase activity (6).

Whether the anti-Jo-1 antibody is involved in the pathogenesis of polymyositis or is an epiphenomenon of the disease process remains to be established. To provide a basis to study the rodent model of coxsackievirus-induced myositis (7), we show that the anti-Jo-1 antibody specifically inhibits not only human HeLa His-tRNA synthetase but also inhibits the same enzyme from rat liver. The anti-Jo-1 antibody does not inhibit the synthetases found in the purified rat liver 18S complex (8,9). Using immuno-

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affinity chromatography, we purified the rat liver His-tRNA synthetase which has a subunit M_r 64,000 and is identical to the Jo-1 antigen.

MATERIALS AND METHODS

Anti-Jo-1 sera were obtained from myositis patients (by F.C. Arnett) previously described (2). Anti-Jo-1 was purified from serum by $(NH4)_2SO_4$ precipitation and DEAE-cellulose chromatography (6). Ouchterlony double diffusion precipitation was performed as in (9). The Jo-1 antigen was assayed by radioimmunoassay. Aliquots of 20 µL were spotted on nitrocellulose discs (Schleicher & Schull BA85). Nitrocellulose discs were incubated at 4 degrees C with shaking in Buffer C (50mM Tris HCl, pH 7.5, 150mM NaCl, 5mM EDTA, 2mg/ml bovine serum albumin, 0.5% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate) for 15 minutes with two changes. Discs were then incubated with anti-Jo-1 serum (100-fold diluted with Buffer C) for 30 minutes, washed with Buffer C for 15 minutes with two changes, and incubated with I^{125}-Protein A (0.3 mCi/mg, 10 µg/ml) in Buffer A for 15 minutes. The discs were washed with Buffer C for 15 minutes with two changes and counted in a Beckman Gamma 5500 counter.

Aminoacyl-tRNA synthetases were prepared and assayed as described (8-10). Histidyl-tRNA synthetase was prepared from HeLa extract according to (6) and purified from rat liver according to Kane et. al. (11) with modifications. All buffers for purification contained 2mM dithiothreitol, 2mM phenylmethyl sulfonyl fluoride, 3mM $MgCl_2$ and 10% (v/v) glycerol. The rat liver postribosomal fraction (8,10) was fractionated with $(NH_4)_2\ SO_4$ and precipitated fractions between 20-40% saturation were used for further purification. All chromatography were carried out at 4 degrees C.

Immunoaffinity gel with immobilized anti-Jo-1 antibody was prepared as follows. Anti-Jo-1 Ig (100 mg) was incubated with 20 mL washed Affi-Gel 10 (Bio Rad) in 30mM potassium phosphate (pH7.0) for 4 hours at room temperature. The coupled gel was washed extensively with 1 M potassium phosphate (pH7.0) and stored in 50% glycerol at -20 degrees C.

Polyacrylamide gel electrophoresis in presence of sodium dodecyl sulfate was performed as described (8).

RESULTS

We observe that the myositis anti-Jo-1 positive sera specifically inhibit HeLa His-tRNA synthetase (data not shown) confirming the results of Mathews and Bernstein (6). The anti-Jo-1 positive sera, which are clinically screened by the Ouchterlony double diffusion technique against rabbit thymus saline extract, remarkably cross-react with rat liver homogenate (Figure 1, inset). No

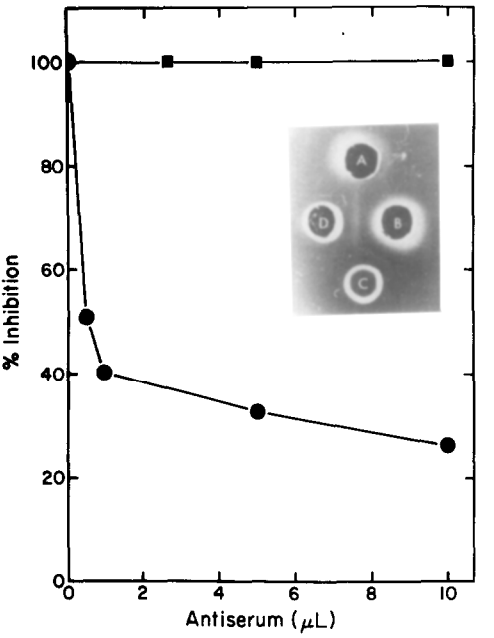


Figure 1: Inhibition of rat liver histidyl-tRNA synthetase by anti-Jo-1 serum. Rat liver post-ribosomal fraction was incubated with increasing amounts of anti-Jo-1 and normal serum (in final volume of 25 μL) for 10 minutes at 0 degrees C. The percent inhibition by anti-Jo-1 serum as compared to control (normal) serum of His-tRNA synthetase (-●-) is shown. Arginyl-,isoleucyl-, leucyl-, lysyl-, and methionyl-tRNA synthetases (-■-) were not inhibited by anti-Jo-1 serum. The inset demonstrate double diffusion precipitation between normal serum (A), anti-Jo-1 serum (B), rabbit thymus extract (C), and rat liver post-ribosomal fraction (D).

precipitin lines were observed with the purified 18S rat liver complex (not shown). These sera specifically inhibit rat liver His-tRNA synthetase activity but not activities specific for Arg, Ile, Leu, Lys and Met in the crude rat liver extract (Figure 1). These findings prompted the pursuit of purified rat liver His-tRNA synthetase as a potential source of easily accessible pure Jo-1 antigen.

Using a slightly modified procedure of Kane et al. (11), we purified rat liver His-tRNA synthetase 2800-fold after three conventional chromatography columns (Table I). The copurification of the Jo-1 antigen with His-tRNA synthetase was determined by a radioimmunoassay using the anti-Jo-1 serum (Table I, see

Table I: Co-Purification of Histidyl-tRNA Synthetase and Jo-1 Antigen from Rat Liver[a]

	Step	Total Protein (mg)	sp.act.[b] (units/mg)	Purification[b]	Jo-1 sp.act.[c] (cpm/mg 10^{-6})	Purification[c]	Ratio[d]
1.	$(NH_4)_2SO_4$ Precipitate	6,240	0.22	1	0.0095	1	1
2.	DEAE-Cellulose	252	3.9	18	0.047	4.8	3.7
3.	Phosphocellulose	27	27.3	124	0.62	62	2.0
4a.	Bio Rex 70[a]	2.0	111	500	--	--	--
4b.	Hydroxyapatite[a]	0.6	618	2,800	6.64	1400	2.0

a. Based on 200g of rat liver. Final steps were either Bio Rex 70 (Step 4a) or Hydroxyapatite (Steb 4b).

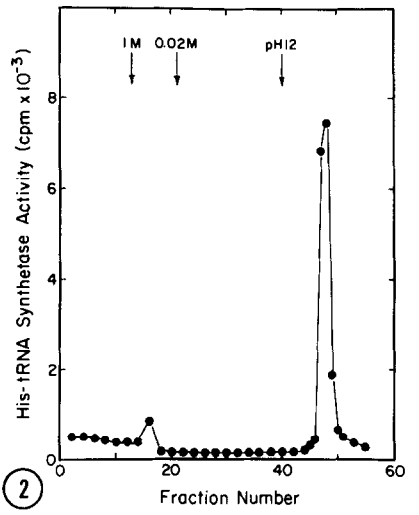
b. Specific activity and purification of histidyl-tRNA synthetase. One unit of histidyl-tRNA synthetase is defined as that amount of activity that incorporate 1 nmol of amino acid in 1 minute under standard assay conditions.

c. Specific activity and purification of Jo-1 antigen as assayed by Proteins A-mediated radioimmuno assay (see Materials and Methods for details).

d. Ratio of purification of histidyl-tRNA synthetase and purification of Jo-1 antigen.

Methods). Chromatography of the phosphocellulose fraction on tRNA-Sepharose (8) or hydroxylapatite columns instead of Bio Rex 70 resulted in similar purification (not shown). The peak activity fractions from the Bio Rex 70 column show polypeptides with M_r 52,000 - 64,000 (Figure 3) determined by molecular weight standards (lane c) also seen in lane b. Further purification on Sephacryl S300 gel filtration column (not shown) resulted in a sharp His-tRNA synthetase activity peak at approximate M_r 100,000 - 150,000. This material showed two polypeptides M_r 52,000 and M_r 64,000 on SDS-polyacrylamide gel electrophoresis (not shown).

Rat liver His-tRNA synthetase was purified by using immuno-affinity chromatography with anti-Jo-1 Ig immobilized on Affi



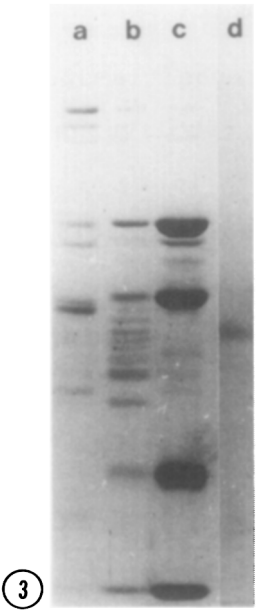


Figure 2: Immunoaffinity chromatography of histidyl-tRNA synthetase using immobilized anti-Jo-1 Ig. All buffers contain 3 mM $MgCl_2$, 2mM dithiothreitol, and 10% glycerol. One mg of Bio Rex 70 purified His-tRNA synthetase was placed on anti-Jo-1-Ig-agarose column (1.0 x 6.0 cm) equilibrated with 0.05M Tris HCl (pH7.5, 25 degrees C). The column was sequentially eluted with 40 ml of 0.05M Tris-HCl (pH7.5), 20 ml of 1M Tris-HCl (pH7.5) and 0.02M Tris-HCl (pH7.5). The antigen was eluted from the column by 0.10M Tris buffer (pH12). Fractions (2 ml) were collected and immediately neutralized with 1M Tris-HCl (pH7.0) and assayed for His-tRNA synthetase activity.

Figure 3: SDS-polyacrylamide gel electrophoresis of purified rat liver 18S aminoacyl-tRNA synthetase complex (lane a), Bio Rex 70 purified His-tRNA synthetase (lane b), standards (lane c), and immunoaffinity chromatography purified His-tRNA synthetase (lane d). Standards (lane c) are phosphorylase b (M_r 92,500) bovine serum albumin (M_r 66,200 ovalbumin (M_r 45,000) and carbonic anhydrase (M_r 31,000).

Gel 10 (Figure 2). The affinity gel can be re-used after thorough washing with the pH 12 buffer and re-equilibration with the starting buffer (Figure 2). Figure 3 (lane d) demonstrates the purified His-tRNA synthetase (M_r 64,000) obtained from re-use of the affinity column. This purified His-tRNA synthetase is markedly labile during storage. Figure 3 (lane a) also demonstrates the polypeptides of the purified rat liver 18S complex used in the double diffusion immunoprecipitation experiments.

DISCUSSION

The myositis-specific anti-Jo-1 autoantibody specifically inhibits rat liver histidyl-tRNA synthetase and that from human HeLa cells (6). We provide direct evidence for the first time in this communication showing that the Jo-1 antigen is His-tRNA synthetase and is not a cofactor in contrast to the indirect evidence reported previously (6). The enzyme purified by immunoaffinity chromatography shows a subunit M_r 64,000. The estimated M_r determined by gel filtration chromatography suggests an α_2 structure for the native enzyme similar to that from rabbit reticulocyte (11). This M_r is comparable to the M_r 150,000 estimated for partially purifed Jo-1 antigen (3) and beef liver His-tRNA synthetase (12). Multiple polypeptides with M_r 52,000 to 64,000 seen in the His-tRNA synthetase preparation from the Bio Rex 70 column may be proteolytic fragments of His-tRNA synthetase. Similar difficulties with proteolysis of the beef liver His-tRNA synthetase was reported (12). The HeLa M_r 50,000 polypeptide immunoprecipitated by anti-Jo-1 antibody was not shown to be His-tRNA synthetase directly (6). Based on our results, this M_r 50,000 polypeptide is likely to be a proteolytic fragment of His-tRNA synthetase.

Cross-reactivity between synthetases from different species indicates that anti-Jo-1 is directed toward conserved structure(s) of the His-tRNA synthetase.

The purified Jo-1 antigen or His-tRNA synthetase will be useful in determining the role of anti-Jo-1 in the pathogenesis of polymyositis and in the intracellular localization of His-tRNA synthetase. Because His-tRNA synthetase is commonly found in free forms and the Jo-1 antigen has been localized both to the nucleus (3) and cytoplasm (5,6), the comparison of its intracellular distribution to that of Met-tRNA synthetase which occurs in

high-M_r complexes (9,13) is warranted for better understanding of the high M_r complexes and the nature of the Jo-1 antigen.

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