Review Letter

Isoelectric focusing and isoelectric points of aminoacyl-tRNA synthetases

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Isoelectric points and isoelectric focusing behaviour of 10 highly purified eukaryotic aminoacyl-tRNA synthetases from 3 sources, *Saccharomyces cerevisiae, Euglena gracilis* and *Phaseolus vulgaris* were examined. The pI-values measured on polyacrylamide gels under native conditions are situated between pH 5.0–7.5. A microheterogeneity was observed for 9 enzymes appearing otherwise homogeneous on gel electrophoresis. A compilation of the isoelectric points of aminoacyl-tRNA synthetases is given and literature data are compared with our experimental results.

Aminoacyl-tRNA synthetase *Isoelectric focusing* *Isoelectric point* *Protein heterogeneity*

1. INTRODUCTION

Among all physicochemical characteristics of a protein, the isoelectric point (pI) is certainly one of the most critical in governing its precipitation and crystallisation. Indeed the solubility of a macromolecule depends upon its isoelectric point (i.e., its net charge) and crystal growth is very often sensitive to the pH of the crystallisation medium [1]. The homogeneity of the protein is a second factor which plays an important role in the crystallisation process and is a prerequisite for obtaining good quality crystals. Thus isoelectric focusing is a very fine technique for examining the charge heterogeneity of purified proteins [2].

In a preliminary step of our attempts of the crystallisation of some enzymes of the family of the aminoacyl-tRNA synthetases (or ligases) (EC 6.1.1), we were looking for data on isoelectric points of these enzymes. Although numerous synthetases have been purified to apparent homogeneity and their M_r and oligomeric structure determined for enzymes originating from more than 50 different organisms (reviewed in [3]), to our surprise only little information on isoelectric points was available. Moreover these data are not easily accessible since they are dispersed in some tables or reference lists (e.g., [4–7]). For these reasons we have collected and discussed data available in the literature and complemented by original results. The new results concern the values of isoelectric points and the behaviour of 10 aminoacyl-tRNA synthetases in isoelectric focusing on polyacrylamide gels. The aminoacyl-tRNA synthetases examined are from 3 eukaryotic sources: the baker's yeast *Saccharomyces cerevisiae*, the green unicellular algae *Euglena gracilis* and the bean *Phaseolus vulgaris*.

2. MATERIALS AND METHODS

2.1. *Enzymes and chemicals*

All the enzymes were prepared in the Laboratoire de Biochimie of the Institut de Biologie Moleculaire et Cellulaire in Strasbourg. Yeast aspartyl-tRNA synthetase was purified as in [8–10]. Yeast valyl-tRNA synthetase, native and proteolysed form [11], arginyl-tRNA synthetase

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[12], methionyl-tRNA synthetase transformant [13] and phenylalanyl-tRNA synthetase [14] were gifts from Drs Kern, Gangloff, Fasiolo and Remy, respectively. Cytoplasmic [15] and chloroplastic leucyl-tRNA synthetases [16] from bean and cytoplasmic [17] and chloroplastic valyl-tRNA synthetases [18] from green algae were gifts from Drs Souciet, Dietrich, Sarantoglou and Imbault.

Acrylamide and methylene–bisacrylamide, especially purified for electrophoresis, were obtained from BDH Chemicals. Urea (ultra pure) was purchased from Schwarz/Mann (Orangeburg NY). Carrier ampholytes (Ampholines, Pharmalytes) of various pH ranges were from LKB and Pharmacia, respectively. Isoelectric point marker proteins were purchased from Serva (protein test mixture 9) and from BDH Chemicals (pI calibration kit).

2.2. *Isoelectric focusing*

Analytical isoelectric focusing was conducted in an LKB Multiphor apparatus connnected to a thermostated water-cooling system and a Pharmacia ECPS 3000/150 power supply. Routinely, thin polyacrylamide slab gels (125 ×160 × 1.0 or 0.5 mm thick), containing 5% (w/v) polyacrylamide with a ratio of methylene–bisacrylamide/acrylamide of 2.7% (w/w) and 2% (w/v) of various carrier ampholyte mixtures and 10% (v/v) of glycerol, were prepared using ammonium peroxodisulphate (0.03%, w/v) and TEMED (0.1%, v/v) for polymerisation. Gels were run with 1 M H_3PO_4 as anolyte and 1 M NaOH as catholyte on the electrode paper strips. Focusing was carried out at 10°C with a pre-run of 15 min at a constant power of 8 W followed by a run of 2 h at 10 W. Samples (1–5 μl) were applied directly onto the gel at 1.5 cm from the cathode. The pH was determined on 5 × 5 × 1.0 or 0.5 mm sections of gel eluted in 1 ml bidistilled water and pH measurements were done at 10°C. On each gel, the isoelectric point of the proteins was estimated by comparison with standard proteins of known pI and from pH gradient curves. The gels were stained for proteins by the classical techniques using Coomassie Brilliant Blue G-250 (Serva), PAGE-Blue 83 (BDH Chemicals) both used at 0.1% (w/v) in a solution containing 25% (v/v) methanol and 10% (v/v) acetic acid, or by the silverstain technique according to [19].

Analytical isoelectric focusing in cylindrical gels containing urea was performed as in [20]. Coomassie blue staining was done as for thin-layer gels.

2.3. *SDS–polyacrylamide gel electrophoresis*

Polyacrylamide gels (11%, w/v, T; 2.7%, w/w, C; 1%, w/v, SDS) were prepared as in [21,22]. The vertical slab gels (160 × 140 × 1 or 0.5 mm) were run overnight at 20°C at constant voltage (150 V). Marker dye was bromophenol blue. Protein samples (~1–5 μg in 15 μl) were mixed with the same volume of twice concentrated dissociating solution [21] and heated for 2 min at 100°C. Proteins were fixed and stained as for isoelectric focusing experiments (see above).

2.4. *tRNA aminoacylation assays*

Gel slices (5 × 1 mm) were eluted in 50 μl 50 mM Tris–HCl buffer (pH 7.2) containing 10 mM glutathione, 120 mM KCl and 0.4 mg/ml bovine serum albumin. Enzyme activity was detected by tRNA aminoacylation assays performed under standard conditions as in [23].

3. RESULTS AND DISCUSSION

One of the aims of this work is the experimental determination of the isoelectric point of several eukaryotic aminoacyl-tRNA synthetases. A prerequisite of such a study is that the proteins to be examined are free of contaminant macromolecules. We estimated by SDS–polyacrylamide gel electrophoresis that the different synthetase preparations had purities of >95%.

The results of isoelectric focusing of the 10 synthetases are summarized in a diagrammatic form in fig.1. Particular banding patterns for 5 enzymes are displayed in fig.2. The experimental data show that the isoelectric points mainly range in the acidic zone from pH 5.0–6.0. This agrees with older observations showing that aminoacyl-tRNA synthetases are mainly contained in a cellular fraction precipitated at pH 5.0 [24]. Methionyl-, arginyl- and especially aspartyl-tRNA synthetase, however, have a more basic behaviour with pI-values ranging from pH ~6.0–7.5.

Unexpectedly, most synthetases exhibit an heterogeneous migration pattern which can comprise numerous bands. In fig.1 the bars represent

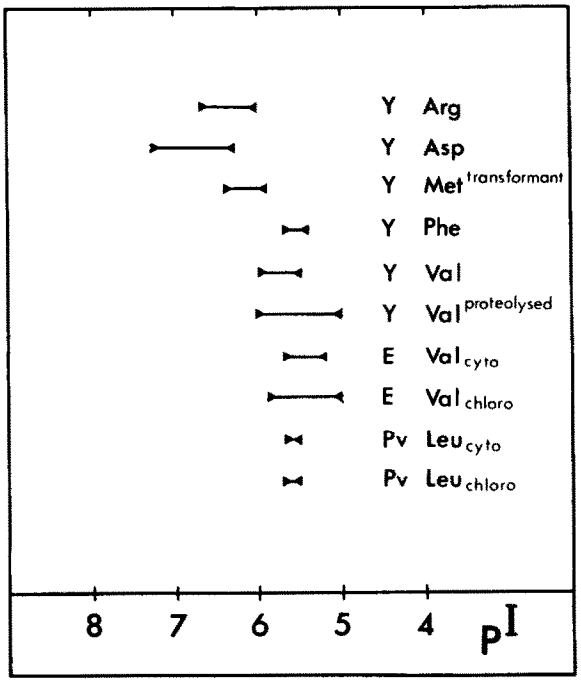

Fig.1. Isoelectric point values of 10 eukaryotic aminoacyl-tRNA synthetases. The results concern freshly prepared enzymes examined by isoelectric focusing in polyacrylamide gels under non-dissociating conditions. pI-Values are given for 10°C. *Abbreviations:* Y, yeast *Saccharomyces cerevisiae*; E, *Euglena gracilis*; Pv, *Phaseolus vulgaris*; cyto, cytoplasmic; chloro, chloroplastic. Enzymes are indicated by the amino acids 3-letter abbreviation.

the pH-range in which the individual protein bands were found. For some synthetases, such as valyl- and phenylalanyl-tRNA synthetase, the number of bands exceeds 10 (see fig.2). Interestingly enough the 3 native valyl-tRNA synthetases examined exhibit similar multiband patterns. Even the transformant cytoplasmic methionyl-tRNA synthetase, which obviously arises from one unique gene, is heterogeneous in isoelectric focusing. This contrasts with the apparent homogeneity of all these enzymes revealed by the classical chromatographic and electrophoretic methods [8–18]. Only the 2 leucyl-tRNA synthetases from *Phaseolus vulgaris* exhibit rather homogeneous pI patterns: for the cytoplasmic enzyme 2 tiny bands migrating closely together are visualised whereas for the chloroplastic protein only a single band is observed after staining with Coomassie blue or silverstain (details in [15]). It should be noted that the isoelectric focusing patterns and the pI-values in fig.1 and 2 are highly reproducible under non-dissociating conditions for the synthetases:

(i) With different enzyme preparations;
(ii) In different pH gradients prepared either with Ampholines or Pharmalytes;
(iii) Whatever the position where the samples are deposited on the gels;
(iv) In either polyacrylamide or agarose gels;
(v) With different staining techniques (section 2).

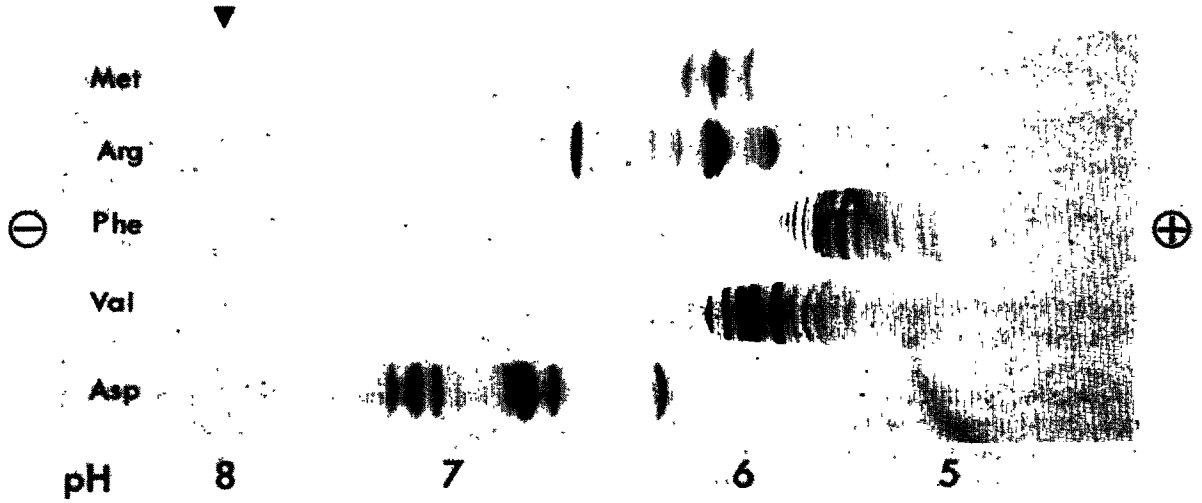

Fig.2. Isoelectric focusing patterns, under native conditions, of 5 cytoplasmic aminoacyl-tRNA synthetases from yeast *Saccharomyces cerevisiae*. Enzyme names are abbreviated by the amino acid. The pH gradient was obtained with a mixture containing 80% LKB Ampholine 5–8 and 20% LKB Ampholine 3.5–10. Proteins were stained by PAGE Blue 83. The arrow indicates the position where the samples (~5 μg protein) were deposited onto the gel.

In the particular case of yeast aspartyl-tRNA synthetase the heterogeneity was also revealed by column chromatofocusing (details to be published elsewhere).

A possible origin of the heterogeneity in isoelectric focusing could arise from more or less important aggregation of the synthetases. This is ruled out by the isoelectric focusing experiments, under denaturing conditions in the presence of 9.5 M urea (fig.3): the heterogeneity always remains, although the distribution of bands is different than under native conditions. This is probably explained by charge changes due to the effect of urea on the proteins conformation, and in the case of oligomeric proteins to the appearance of new bands due to the dissociation of subunits (disappearance of some bands after dissociation of aggregates, however, is not excluded).

These observations raise the question of the possible origin of the observed charge heterogeneity. Although it cannot be excluded that some minor bands can originate from contaminating proteins (in any case these contaminants are $\leqslant 5\%$ of the material) most of these bands, especially the major ones, correspond to aminoacyl-tRNA synthetase. In the case of arginyl-, aspartyl-, phenylalanyl- and valyl-tRNA synthetase from yeast, enzymatic activity was found in all bands, even in the minor ones, of the migration patterns (not shown). As has been discussed for numerous proteins (e.g., [2,25,26]) the charge heterogeneity observed for the synthetases might be due, for example, to covalently bound carbohydrates, lipids, metal ions, post-translational modifications and proteolysis, leading to multiband isoelectric focusing patterns. In a few cases, binding to the protein of ampholytes or other compounds present in the gel have been reported [27]; such an effect can also perturb the migration patterns.

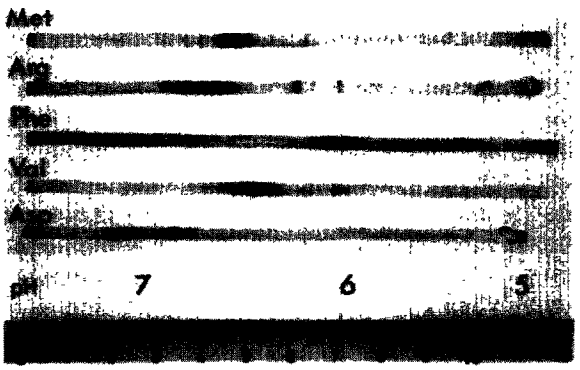

Fig.3. Isoelectric focusing patterns, under denaturing conditions, in the presence of urea, of 5 cytoplasmic aminoacyl-tRNA synthetases from yeast. The names of the enzymes are abbreviated by the amino acid. The cylindrical gels were prepared according to [20]. The pH gradient and other technical data were as in fig.2. The pH-values given in the figure were corrected for temperature and the urea-induced effect [54,55] so that data can be compared with those in fig.1 and 2.

To have a general view about isoelectric points for prokaryotic and eukaryotic aminoacyl-tRNA synthetases, we have compared our own observations with those available in literature. Table 1 summarizes our present knowledge in this field. The collected data cover 40 different aminoacyl-tRNA synthetases originating from 3 prokaryotic sources (*E. coli, Bacillus* and *Salmonella*) and 10 eukaryotic sources (yeasts, protozoa, algae, higher plants and mammals including human). They correspond, however, only to 11 different amino acids. The pI-values seem to be more acidic in prokaryotes compared to eukaryotes. This would agree with the different behaviour observed in column chromatography, for example between the yeast and *E. coli* synthetases [9,50]. Proteolysed forms of synthetases can be more acidic (yeast valyl- and *E. coli* methionyl-tRNA synthetase) or more alkaline (wheat methionyl-tRNA synthetase) than the native proteins. Only one datum is available for a mitochondrial synthetase, the leucyl-tRNA synthetase from the protozoan *Tetrahymena pyriformis*; this enzyme is much more acidic than the corresponding cytoplasmic one, reflecting structural differences between these two aminoacyl-tRNA synthetases [33].

Numerous enzymes listed in table 1 have been described as migrating as a single population of molecules in focusing experiments (e.g., [28,31–33,35,36,39,40,42,48]). Considering our own results, showing an heterogeneous behaviour of synthetases, one may question the validity of some of these results, especially since migration patterns are not always shown. A likely explanation of the discrepancy could be the use of less efficient resolving methods in earlier experiments. Heterogeneity in isoelectric focusing of pure synthetases has nevertheless been observed in several

Table 1

Compilation of isoelectric point values of aminoacyl-tRNA synthetases

Aminoacyl-tRNA synthetase[a]	Organism	Organ and/or cellular compartment	M_r ($\times 10^{-3}$)	Quaternary structure and M_r ($\times 10^{-3}$) of subunits	pI[b]	Θ[c]	No. of enzyme populations[d]	Ref.
Arg	*Saccharomyces cerevisiae*		73	α	6.0–6.7; 6.2	10°C	5	Here
Asp	*Saccharomyces cerevisiae*		120	α_2	6.3–7.3; 6.7,6.8	10°C	9	Here
Glu	*Escherichia coli*		102	$\alpha\beta$ $\alpha = 56$ $\beta = 46$	4.5, 5.5 (*)	(3°C)	1	[28]
	Bacillus subtilis		65.5	α	4.3	n.g.		[29]
His	*Salmonella typhimurium*		80	α_2	5.9	(4°C)		[30]
	Rabbit	Reticulocyte cytosol	122	α_2	5.1	n.g.	1	[31]
Leu	Baker's yeast		120	α	7.0	(4°C)	1	[32]
	Tetrahymena pyriformis	Cytoplasm	100	α	8.7	(4°C)	1	[33]
		Mitochondria	100	α	6.5	(4°C)	1	[33]
	Pisum sativum	Cotyledon			4.1, 5.3, 6.9	n.g.	3	[34]
	Phaseolus *vulgaris*	Cytoplasm			5.5–5.7	10°C	2	Here and [15]
		Chloroplast	122	α	5.5–5.7	10°C	1	Here
Met	*Escherichia coli*	Native	170	β_2	5.0	n.g.	1	[35]
		Trypsinized	60	α	4.8	n.g.	1	[35]
	Bacillus brevis		100		4.4 (*)	(4°C)	1	[36]
			200		5.0 (*)	(4°C)	1	[36]
	Saccharomyces cerevisiae	(Transformant)			5.9–6.3	10°C	3	Here
	Wheat germ	Form A	105	β	6.0 (*)	(4°C)		[37]
		Form B	70	α	5.85 (*)	(4°C)		[37]
	Wheat germ	Cytoplasm	165	β_2	5.1, 5.5, 6.1, 6.9, 7.8	n.g.	5	[35]
		Proteolyzed	70	α	6.1	n.g.		[35]
	Wheat	Chloroplast	75	α	5.5	n.g.	1	[35]

(continued)

Table 1

Aminoacyl-tRNA synthetase[a]	Organism	Organ and/or cellular compartment	M_r ($\times 10^{-3}$)	Quaternary structure and M_r ($\times 10^{-3}$) of subunits	pI[b]	Θ[c]	No. of enzyme populations[d]	Ref.
Phe	*Saccharomyces cerevisiae*		270	$\alpha_2\beta_2$ $\alpha = 73$ $\beta = 63$	5.4–5.7	10°C	*n*	Here
	Rat	Liver			7.0 (*)	1°C		[38]
Ser	*Escherichia coli* B		103	α_2	6.1–6.2 (in urea)	n.g.	1	[39]
	Escherichia coli K-12		100	α_2	3.9	n.g.	1	[40]
Thr	*Saccharomyces carlbergensis*		160	α_2	6.2, 6.3	n.g.	2	[41]
	Rat	Liver	170	α_2	6.4	0°C	1	[42]
Trp	*Escherichia coli* B		74	α_2	6.2	0°C		[43]
	Bacillus stearothermophilus	Cytoplasm	70	α_2	4.5–5.0	n.g.	*n*	[44]
	Human	Lymphocyte (chronic leukemia)	90	(α_2)	5.2–5.3	0°C		[45]
		Placenta	120	α_2	5.2	0°C		[46]
		Skin	100	α_2	5.2	n.g.		[47]
Val	*Escherichia coli*	Cytoplasm						
		Normal	112	α	4.8 (*)	n.g.	1	[48]
		Phage infected	112	α	4.8 (*)	n.g.	1	[48]
	Saccharomyces cerevisiae	Native	130	α	5.5–6.0	10°C	*n*	Here
		Proteolysed	120	α	5.0–6.0	10°C	*n*	Here
	Euglena gracilis	Cytoplasm	126	α	5.2–5.7	10°C	*n*	Here
		Chloroplast	126	α	5.0–5.9	10°C	*n*	Here
Glu, Ile, Leu, Lys, Met complex	Rat	Liver microsome			5.7, 6.2, 6.5, 7.0	(0–2°C)	1	[49]

[a] Aminoacyl-tRNA synthetases are given by the 3-letter abbreviation of the corresponding amino acid

[b] Isoelectric point; in the case of several enzyme populations the isoelectric point of the major one(s) is underlined or a pI interval is given. (*) indicates that the values derived from column isoelectric focusing experiments. Unless otherwise indicated all values are from polyacrylamide gel isoelectric focusing under non-dissociating conditions

[c] Temperature (in °C) at which the pH was measured; when this data is not available, the temperature of the focusing experiments is given in brackets; n.g. means that no temperature indication is given.

[d] *n* means several enzyme populations

instances [34,35,41,44], in agreement with our data. These observations could be related to multiple forms of synthetases found on different chromatographic supports [51].

The isoelectric points is one of the most important parameters which is involved in the crystallisation of macromolecules [1]. It is thus interesting to compare the pH of crystallisation and the pI of the protein to be crystallised. A good correlation exists for yeast aspartyl-tRNA synthetase which exhibits pI of 6.3–7.3 and crystallises in this range [10]. In that case nucleation is favoured by the reduced solubility of the synthetase around its isoelectric point. The overall electric charge neutrality of a macromolecule, however, is not the only parameter responsible for its precipitation. Therefore, it is not surprising that the tryptic fragment of methionyl-tRNA synthetase from *E. coli* can be crystallised at a pH different from the pI [35,52].

An alternative use of isoelectric focusing in crystallisation experiments is related to the high resolving power of this technique which easily detects microheterogeneity in macromolecules. Thus improved purification methods by isoelectric focusing can lead to proteins able to crystallise, either more easily or in a more ordered form. Such improvements of the quality of protein crystals have actually been described for several proteins [53] and experiments in that line are underway in the case of yeast aspartyl-tRNA synthetase.

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