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Catalytic Activity of Aminoacyl tRNA Synthetases and Its Implications for the Origin of Life I. Aminoacyl Adenylate Formation in Tyrosyl tRNA Synthetase

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Summary. The changes in the catalytic activity resulting from amino acid substitutions in the active site region have been theoretically modeled for tyrosyl tRNA synthetase (Tyr-RS). The catalytic activity was calculated as the differential stabilization of the transition state using electrostatic approximation. The results indicate that charged residues His45, His48, Asp78, Asp176, Asp194, Lys225, Lys230, Lys233, Arg265, and Lys268 play essential roles in catalysis of aminoacyl adenylate formation in Tyr-RS, which is in general agreement with previously known experimental data for residues 45, 48, 194, 230, and 233. These catalytic residues have also been used to search for sequence homology patterns among class I aminoacyl RSs of which HIGH and KMSKS conserved sequence motifs are well known. His45 and His48 belong to the HIGH signature sequence of class I aminoacyl tRNA synthetases (aRSs), whereas Arg265 and Lys268 can constitute a part of the KMSKS charge pattern. Lys225, Lys230, and Lys233 may be part of the conservative substitution pattern [HKR]-X(4)-[HKR]-X(2)-[HKR], and Asp194 is part of the new GSDQ motif. This demonstrates that the three dimensional charge distribution near the active site is an essential feature of the catalytic activity of aRS and that the theoretical technique used in this work can be utilized in searches for the catalytically important residues that may provide a clue for a charge residue pattern conserved in evolution. The appearance of patterns I–IV in Arg-, Gln-, Met-, Ile-, Leu-, Trp-, Val-, Glu-, Cys-, and Tyr-RS indicates that all these enzymes could have the same ancestor.

Key words: Aminoacyl tRNA synthetase — Catalytic activity — Transition state stabilization — Sequence motif — Quantum chemical study — Enzyme evolution — Aminoacyl adenylate formation — Signature sequences

Introduction

The foundation of the genetic code rests upon the specificity of matching amino acids with tRNAs via mediation of aminoacyl tRNA synthetases (aRSs) (de Duve 1988; Schimmel 1989). Despite great diversity in size and specificity, all present-day aRSs share two common catalytic functions, the formation of aminoacyl adenylate and the formation of aminoacyl tRNA. Because the chemical reactions involved in those steps are common to all amino acids, the catalytic mechanisms are likely to be identical. The functional identity of the various aRSs suggests a common ancestral aRS, which, presumably, first acquired the structural characteristics needed for the catalytic functions and only subsequently evolved specific recognition for the amino acids and tRNAs.

In our continuing effort to characterize the ancestral translational apparatus (Rein et al. 1987), we have modeled the chemical and structural elements required for the catalysis of aminoacyl adenylate formation. Such elements might be preserved during evolution and appear as conserved sequence motifs

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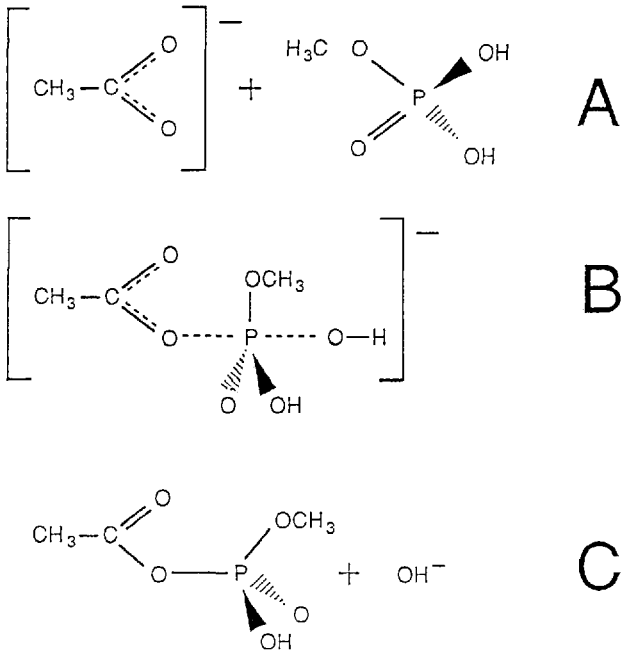

Fig. 1. Model reactants for aminoacyl adenylate formation in aRS. **a** Model substrate; **b** model transition state; and **c** model product.

in the present day aRS. Thanks to earlier extensive site-directed mutagenesis (SDM) studies (Leatherbarrow et al. 1985, 1987; Lowe et al. 1985; Fersht 1987; Fersht et al. 1988; Garcia et al. 1990), the catalytic importance of various tyrosyl tRNA synthetase (Tyr-RS) residues is well characterized, allowing for comparison of theoretical and experimental results and for verification of the theoretical approach.

The catalytic residues would exert their activity by lowering the activation barrier for the reaction proceeding within the active site of the enzyme. Electrostatic differential stabilization energies for transition state resulting from amino acid substitution can be calculated within an approach based on quantum chemical theory of intermolecular interactions (Sokalski 1985). Using the atomic multipole approximation in the above approach, the effects of all possible amino acid substitutions on the model reaction were examined for the Tyr-RS active site residues including some more distant charged amino acids located within a 22-Å radius from the reaction center. It is understandable that such an approximate approach cannot provide quantitative estimates of changes in free energy of activation with precision comparable to experimental data. Due to the electrostatic character of this model our study focuses on a theoretical search for charged residues contributing to Tyr-RS catalytic activity. The theoretical prediction of catalytic residues offers a complementary approach to identification of those residues by SDM. Comparison of conserved charged patterns identified by sequence analysis with the experimentally or theoretically identified catalytic residues lends insight into the evolutionary significance of nature and the origin of the catalytic mechanism.

Methodology

Modeling of Reactants. The first reaction catalyzed by Tyr-RS is formation of tyrosyl adenylate (Tyr-AMP) from tyrosine (Tyr) and ATP.

$$\text{Tyr} + \text{ATP} \xrightarrow{\text{Tyr-RS}} \text{Tyr-AMP} + \text{P-P}_i \qquad (1)$$

Although the crystallographic data on the Tyr-RS complex with tyrosinyl adenylate (a Tyr-AMP analog) serve as a reference to the product state of this reaction (Monteilhet and Blow 1978; Bhat et al. 1982; Brick et al. 1989), more structural detail would be desirable for a comprehensive theoretical study of the catalytic activity of Tyr-RS. Specifically, precise geometries of the substrate, transition, and product state complexes including positions of hydrogen atoms are essential for theoretical studies (Krauss and Garmer 1990).

The corresponding structures have been modeled here using ab initio molecular orbital theory. Because the size of the reactant is so large, we have modeled only the elements directly involved in the amino acid and ATP reaction (Fig. 1). The amino acid and ATP are modeled by acetate anion and methylphosphate, respectively. First, we optimized the structures of model substrate (acetate anion and methylphosphate: Fig. 1a) and model product (Fig. 1c) within the 3–21G basis set. The approximate structure of the transition state (Fig. 1b) was obtained with the linear synchronous transit approach (Halgren and Lipscomb 1977). ATP negative charge is localized mostly in β and γ phosphate groups, which are counterbalanced by the bound Mg^{2+} ion in Tyr-RS (Garcia et al. 1990). Therefore the use of a neutral model compound for the ATP molecule appeared justified. In addition, it was assumed that the interactions of the catalytic residues with the neglected part of reactants were similar for both the substrate and transition states and therefore do not affect significantly the relative differences in the activation barrier lowering Δ. Because the tyrosine sidechain is not included, the calculation does not reflect any specific tyrosine-binding properties and thus represents the more general features of aminoacyl tRNA synthetases.

Theoretical Determination of Catalytic Residues. The activation barrier for enzyme-catalyzed reactions can be modulated by substituting single amino acids. Such modifications can be introduced by SDM, and the effects can be measured as a reaction rate acceleration k_G/k_E, where k denotes the rate constant. This effect of amino acid substitutions on enzyme reaction kinetics can also be rapidly estimated by using a simple theoretical model (Sokalski 1985).

The lowering of the activation barrier, $\Delta = B_E - B_G$, is defined as the difference of barrier height in the presence of any environment B_E, and in the absence of environment or gas phase B_G. Δ can be alternatively expressed as the difference of two interaction energies $\Delta E_{TC.E}$ and $\Delta E_{SC.E}$. The first term, $\Delta E_{TC.E}$, denotes the interaction energy of a transition complex (TC) with the environment (E) and the latter term, $\Delta E_{SC.E}$, indicates the interaction energy of a substrate complex (SC) with the environment (E).

$$\Delta = B_E - B_G = \Delta E_{TC.E} - \Delta E_{SC.E} \approx RT \ln(k_G/k_E) \qquad (2)$$

In this approach, using the terms analogous to components of the intermolecular interaction energy as defined within exchange perturbation theories, Δ is constructed from electrostatic multipole (EL,MTP), electrostatic penetration (EL,PEN), exchange (EX), induction (IND), and dispersion (DISP) components.

$$\Delta = \Delta E^{(1)}_{EL,MTP} + \Delta E^{(1)}_{EL,PEN} + \Delta E^{(1)}_{EX} + \Delta E^{(R)}_{IND} + \Delta E^{(2)}_{DISP} \quad (3)$$

Accumulating evidence indicates that Δ can be realistically represented by the electrostatic term alone (Sokalski 1985; Hwang and Warshel 1987; Naray-Szabo 1988; Sokalski et al. 1989; Bajarath et al. 1991).

$$\Delta \approx E_{EL,MTP}(TC.E) - E_{EL,MTP}(SC.E) \quad (4)$$

This is consistent with the observation that the driving force for the observed differences in catalytic activity can be attributed to the charge redistribution of the reactants along the reaction pathway from substrates to transition state and the change of corresponding interactions with the environment (Sokalski 1985; Hwang and Warshel 1987; Bajorath et al. 1991).

The value of the electrostatic contribution $\Delta E_{EL,MTP}$ can be rapidly evaluated by using the atomic multipole expansion. In this work atomic multipoles have been generated from the ab initio wave functions calculated for the substrates (acetate anion and methylphosphate complex) and transition state model, following the procedure of Sawaryn et al. (1989).

Due to the perfect additivity of the electrostatic interactions, the environment can be represented as a superposition of its structural subunits i.e., amino acids, using the database of cumulative atomic multipole moments for amino acids (Sokalski et al. 1989).

Results and Discussion

In this work we examined the effect of all possible amino acid substitutions for charged Tyr-RS residues located up to 22 Å away from the active site and for some neutral residues within 9 Å from the reactants that were subjected to earlier SMD experimental studies.

Although the environmental effects are neglected in our calculations of Δ, the vacuum approximation was successfully applied to reproduce experimentally observed trends of pK_a shifts of several histidines in mutated subtilisins (Sokalski et al. 1989).

Table 1 contains one-letter codes for substituted amino acids arranged by decreasing catalytic activity measured by the corresponding Δ values. The native sequences (from Tyr-RS of *Bacillus stearothermopilus*) have been underlined. For sake of comparison Table 1 summarizes also known SDM experimental results related to identity and magnitude of activity of Tyr-RS catalytic residues. As shown in Table 1, our calculations generally agree with available experimental data indicating that His45, His48, Asp194, Lys230, Arg265, and Lys268 are catalytically important residues.

It is interesting to note that several of the amino acid substitutions predicted to be the most catalytically active match exactly the native Tyr-RS sequences as found in *B. stearothermopilus, Escherichia coli,* and *Bacillus caldotenax* (His45, His48, Arg100, Glu152, Lys225, Arg265) or are close to perfect matching, i.e., belong to the three most catalytically active substitutions (Tyr34, Cys35, Asp78, Pro79, Glu85, Glu166, Gln173, Asp176, Gly192, Ser193, Asp194, Gln195, Lys230, Lys233, and Lys268).

Mismatching of some of the predicted substitutions with the native sequence for Asp38, Thr40, Asp42, Tyr51, Lys82, Lys83, Arg86, and Asp227 can be interpreted as follows:

1) The residue may play a different role during AMP formation. For example, it can be responsible for catalytic activity during the second reaction, play a structural role, or secure binding of the substrates.

2) The proposed orientation of the reactants in the active site may be ambiguous, in particular with respect to the positioning of the hydrogen atoms. In this study the location of tyrosinyl adenylate inhibitor served as a template (Brick et al. 1989), which, however, may not exactly correspond to that of the native reactants.

3) Some residues, especially Asp38, are located too close to the reactants, where multipole approximation may not be applicable. Alternatively, it is possible that these residues may not be essential for the catalysis, serving some other purposes such as preserving substrate specificity or structural stability.

It is interesting to note that residues Asp33 and Asp42 (for which Δ mismatch has been observed) are not universally preserved in other tRNA synthetases.

4) All amino acid substitutions have been considered without allowing for possible structural reorganization. Such effects can be modeled only with much more elaborate and costly molecular dynamics calculations.

Table 1 shows clearly that both SDM experiments and our theoretical calculations indicate the importance of charged residues in the aRS catalytic activity. The essential role played by ionic residues in the catalytic activity of other enzymatic systems has also been shown in recent quantum chemical studies on carbonic anhydrase (Krauss and Garmer 1990) and dihydrofolate reductase (Bajorath et al. 1991). Of course neither are all of the catalytic residues charged nor do all of the charged residues play a role in substrate binding. Nevertheless, it is interesting to point out that the majority of charged residues in the native Tyr-RS examined in this study (14 out of 19) belongs to the group of preferred residues among the 20 possible amino acid substitutions.

In order to examine the evolutionary significance of the catalytic residues identified by experiment and by our calculations, we have scanned known sequences of aminoacyl tRNA synthetases from the NBRF Protein Identification Database— Release 26 (George et al. 1986) in search of conserved patterns involving charged residues. Specifically, we searched for the occurrence of charge patterns similar to those found in residues 78–86, 192–195, 225–233, and 265–270 of Tyr-RS, which allow for substitutions among similarly charged residues ($K^+/R^+/H^+$ or $D^-/$

Table 1. One-letter codes for substituted amino acids arranged according to the decreasing catalytic activity masured by Δ valves

Native residue[a]		R (Å)[b]	Theoretically determined catalytic residues (most active → least active)	Experimentally determined catalytic residues		
				Residues	References[c]	Relative catalytic activity[d]
34	Y	12.9	EDS Y VLNMPFAGTCRWKHIQ	Y		0
35	C	9.4	R C DQWTPNLAFMYIVGSHEK	C	a	++
38	D^-	4.6	NYVSQGECRL D FPIWAHTMK			
40	T	6.6	REDFQWICPAYSN T GLVHMK	T	b	++++
42	D^-	14.5	HRKMGITAYFWVSLQPCNE D			
45	H^+	10.5	H RKYQCWPMAFNTSGEIVLD	H	b	++++
48	H^+	7.5	H WKRNLPSVCGIQMFYTAED	H	a, c	+++
51	T	8.7	REQDYWCMFAIPLGSNHV T K	Y	c, d	
78	D^-	6.3	EQLM D FYVSGWACPTINHKR			
79	P	11.3	DE P NCAQLSVFTYWGIMKRH			
82	K^+	10.0	EDWAVLTGFYSIPMQWCRH K	K	e	++
83	K^+	15.4	DECQNPTYFALWMSIVGR K H			
85	E^-	16.0	D E CWQTIAMYFPNGLRVSKH			
86	R^+	11.6	EDFVLAYGSWIMTPCQNH R K	R	e	+++
100	R^+	14.7	R HKQCFTAWPILMVGNSYDE			
152	E^-	15.1	E DNVYGMPSIALTWFCQKHR			
166	E^-	13.5	D E CWQIGMNTAYPFVLSRKH			
173	Q	10.0	ED Q WMYFAVGSCIPLTNRHK			
176	D^-	12.7	E D LVAFGSIMWTPYCQNHRK			
192	G	8.5	KVLN G TMISAPDWCRHEQFY			
193	S	11.6	DENP S LYQVFACWTMIGKHR			
194	D^-	8.2	EY D FSNVGLMAPITWQCKHR	D	f	++++
195	Q	7.6	WEDI Q ACYFKMLGPSVTNRH			
225	K^+	20.0	K HRNSPVLWIGMCFTAYQDE			
230	K^+	15.6	RH K CTAFQWLYPMVINESGD	K	e	++++
233	K^+	16.0	H K RWSVGILNMPFYAQCTED	K	e	
265	R^+	21.7	R HKQCWTFAMIYLPGVSNDE			
268	K^+	18.2	HR K GWMCIQYAFTVDSLPNE			

[a] Native sequences (Tyr-RS from *B. stearothermopilus*) have been underlined

[b] The distance between the Pα atom of the reactant and the Cβ of the corresponding native residue

[c] a, Wells et al. 1985; b, Leatherbarrow et al. 1985; c, Lowe et al. 1985; d, Carter et al. 1984; e, Fersht et al. 1988; f, Fersht 1987

[d] Relative catalytic activity estimated from experiment (Fersht 1987) as a difference of apparent stabilization energies of transition state and substrates by the respective side chain. Key to symbols: 0 = 0.0–0.5, + = 0.5–1.0, ++ = 1.0–1.5, +++ = 1.5–2.0, ++++ = >2.0 kcal/mol

E^-). The resulting conserved charge patterns for 25 aRSs are shown in Table 2.

In class I aRSs, the existence of two conserved motifs, HIGH (Webster et al. 1984) and KMSKS (Hountondji et al. 1986), is well established (Schimmel 1991). The importance of charged residues in HIGH motif I for Tyr-RS catalysis was demonstrated by SDM experiments for His45 (Leatherbarrow et al. 1985) and His48 (Wells et al. 1985). Our calculation also confirms the importance of His45 and His48 for the Tyr-RS system. For the Met-RS system, Mechulam et al. (1991) recently showed that the substitution of Lys335 in the KMSKS motif by Ala or Gln resulted in a significant decrease in its catalytic activity. For the Tyr-RS system it was shown that the replacements of Lys230 and Lys233 in Tyr-RS (*B. stearothermopilus*) resulted in a dramatic loss of Tyr-RS catalytic activity (Fersht et al. 1988). This led Breton et al. (1990) and Eriani et al. (1991) to propose that KFRK in Tyr-RS corresponds to the KMSKS motif. Our calculation demonstrated that Lys230 and Lys233 exert the maximum catalytic activity toward reaction (1) and the two positively charged residues Arg265 and Lys268 also contribute to the catalytic activity of Tyr-RS. It turns out then that a similar pattern of charged residues +XX+ is conserved among the Tyr-RSs, and this pattern may correspond to the KMSKS motif found in other class I aRSs.

In the Tyr-RS system (*B. stearothermopilus*), a cluster of positively charged residues, Lys225, Lys230, and Lys233, exert maximum catalytic ac-

Table 2. Sequence patterns from several class I aminoacyl tRNA synthetases

Pattern:	I			II			III					IV			
Predicted pattern:	+		+		−		+		+		+	+			
Tyr-RS B.S.	^{45}H	IG	H[a]	^{192}GS	D	Q[d]	^{225}K	ADGT	K	FG	K[c]	^{265}R	YL	K	Y[c]
Tyr-RS B.C.	^{45}H	IG	N[a]	^{192}GS	D	Q[d]	^{225}K	ADGT	K	FG	K[c]	^{265}R	YL	K	Y[c]
Tyr-RS E.C.	^{47}H	LG	H[a]	^{197}GS	D	Q[d]	^{229}K	ADGT	K	FG	K[c]	^{269}R	FL	K	F[c]
Tyr-RS N.C.	^{110}H	VG	H[a]	^{270}GS	D	Q[c]	^{318}D	SSGA	K	FG	K[c]	^{362}K	LF	T	F[c]
Met-RS E.C.	^{21}H	LG	H[a]	^{68}TP	E	Q[d]	^{142}K	GTCP	K	C	K[c]	^{332}K	MS	K	S[b]
Met-RS S.C.	^{212}H	LG	N[a]	^{221}SA	D	I[d]	^{262}R	QLCD	K	YH	K[c]	^{525}K	FS	K	S[b]
Cys-RS E.C.	^{37}H	IG	H[e]	^{227}GS	D	L[c]	^{55}R	FLGY	K	L	K[c]	^{266}K	MS	K	S[e]
Ile-RS E.C.	^{64}H	IG	H[a]	^{562}GS	D	Q[d]	^{431}R	AQSL	K	EI	K[c]	^{602}K	MS	K	S[b]
Ile-RS S.C.	^{54}H	YG	H[a]	^{562}GL	D	Q[d]	^{168}K	GQVY	R	GF	K[c]	^{602}K	MS	K	S[d]
Leu-RS S.D.	^{63}H	IG	H[a]	^{300}KS	D	Q[c]	^{204}K	QWFL	G	IT	K[c]	^{646}K	MS	K	S[c]
Leu-RS E.C.	^{49}H	MG	H[a]	^{302}GV	D	T[c]	^{537}H	LLYF	R	FF	H[c]	^{619}K	MS	K	S[d]
Leu-RS N.C.	^{91}H	AG	H[c]	^{394}GS	D	L[c]	^{485}K	INSP	K	DA	K[c]	^{757}K	MS	K	S[c]
Trp-RS E.C.	^{17}T	IG	N[a]	^{147}GE	D	Q[d]	^{106}R	MTQF	K	D	K[c]	^{195}K	MS	K	S[b]
Trp-RS B.S.	^{15}T	IG	N[a]	^{144}GE	D	Q[d]	^{104}R	MTQF	K	E	K[c]	^{192}K	MS	K	S[b]
Trp-RS B.T.	^{159}H	VG	H[c]	^{298}AI	D	Q[c]	^{235}F	YKVV	K	IQ	K[c]	^{337}K	MS	A	S[c]
Trp-RS S.C.	^{48}H	LG	N[a]	^{196}GD	D	Q[d]	^{142}R	MTQW	K	S	K[c]	^{244}K	MS	K	S[b]
Val-RS E.C.	^{49}H	MG	H[a]	^{508}GF	D	I[d]	^{165}K	EDLI	Y	RG	K[c]	^{554}K	MS	K	S[d]
Val-RS B.S.	^{56}H	LG	H[a]	^{485}GY	D	I[d]	^{236}H	PDDE	R	YK	H[c]	^{525}K	MS	K	S[d]
Val-RS S.C.	^{196}H	IG	H[a]	^{663}GW	D	I[d]	^{240}K	QIWA	K	DR	K[c]	^{702}K	MS	K	S[d]
Glu-RS E.C.	^{16}H	VG	G[a]	^{200}GE	D	H[d]	^{218}K	APVP	V	YA	H[c]	^{237}K	LS	K	R[d]
Glu-RS R.M.	^{19}H	VG	T[c]	^{216}GE	E	W[d]	^{190}K	ADGM	P	TY	H[c]	^{253}K	LS	K	R[d]
Gln-RS E.C.	^{40}H	IG	H[c]	^{125}TP	E	Q[c]	^{141}K	NSPY	R	P	R[c]	^{267}V	MS	K	R[d]
Gln-RS H.S.	^{44}H	IG	H[c]	^{128}PA	E	Q[c]	^{167}H	SCCL	R	A	K[c]	^{265}V	LS	K	R[c]
Gln-RS S.C.	^{265}H	IG	H[c]	^{472}LC	D	Q[c]	^{421}R	VLNA	P	HP	R[c]	^{495}V	LS	K	R[c]
Arg-RS E.C.	^{129}H	VG	H[c]	^{477}DE	E	Q[c]	^{324}R	YETL	H	AD	R[c]	^{548}R	NS	R	L[c]

Abbreviations: B.C., *Bacillus caldotenax*; S.D., *Saccharomyces douglassi*; E.C., *Escherichia coli*; B.S., *Bacillus stearothermopilus*; S.C., *Saccharomyces cerevisiae*; N.C., *Neurospora crassa*; R.M., *Rhizobium meliloti*; B.T., *Bos taurus*; H.S., *Homo sapiens*

[a] Burbaum et al. 1990

[b] Hountondji et al. 1986

[c] New sequences identified in NBRF database following theoretical results obtained in this work

[d] Breton et al. 1990

[e] Eriani et al. 1991

tivity toward reaction (1) in agreement with the above-mentioned experiments. In addition, a similar cluster of positively charged residues found in the Met-RS system, Lys142–Lys147–Lys149, was shown to play some role (Meinnel at al. 1990). The pattern of three positively charged residues [HKR]-4X-[HKR]-2X-[HKR] turned out to be conserved among most of 25 aRSs examined here (Table 2, Pattern III). However, the existence of this pattern as well as whether the KFGK sequence from Tyr-RS is a part of this pattern or a part of the KMSKS motif cannot be definitely decided until the calculations for other aRSs can be performed.

Both the experiment (Fersht 1987) and our calculation indicated that there is a significant contribution of Asp194 to the catalytic activity of Tyr-RS. The residues surrounding Asp194 (GSDQ) overlap to some degree with sequences found in several other aRSs (Breton et al. 1990), but due to poor sequence matching this conserved substitution pattern II [G-K-A-D-S-T-L]-X(l)-[D-E]-[Q-I-H-W-T-L] was not yet claimed as a motif.

In addition to the charged sequences I–IV, there are a few isolated negatively charged residues, namely Asp78 and Asp176, which contribute to the lowering of the activation barrier. In the Tyr-RS crystal structure those residues are situated in the same area as Asp194 in the GSDQ motif. Unfortunately, it is very difficult to examine the conservation of a single residue without additional sequence matching or structural data. Thus, evaluation of the significance of those negatively charged residues in the evolution of the synthetases will have to wait until more three-dimensional structures become available.

In the recently proposed partitioning of tRNA synthetases into two classes (Eriani et al. 1990), class II RSs, (GlY-, Ala-, Pro-, Ser-, Thr-, Asp-, Asn-, His-, Lys-, Phe-RS) are characterized by the occurrence of three motifs. However, for class I (Glu-, Gln-, Arg-, Val-, Ile-, Leu-, Met-, Tyr-, Cys-, Trp-

RS), only the two known motifs (HIGH and KMSKS) have been clearly characterized, despite the existence of other overlapping sequences for part (but not all) of class I RSs (Breton et al. 1990; Eriani et al. 1991). Our results indicate that class I RSs may in fact have more conserved motifs (patterns) that are essential for their catalytic activity.

Conclusions

Theoretical electrostatic differential stabilization energies obtained for charged residues of tyrosyl tRNA synthetase indicate that His45 and His48 (*B. stearothermophilus* Tyr-RS numbering) from the signature sequence (motif I), positively charged residues at position 225, 230, and 230 (pattern III), positively charged residues 265 and 268 (pattern IV), as well as Asp194 from the GSDQ sequence (pattern II) are important for catalytic activity for aminoacyl adenylate formation. As these results are in reasonable agreement with known experimental data summarized in Table 1, we concluded that our approach can identify catalytically important residues. This can be useful in situations where less SDM experimental data are at hand. The above-mentioned residues may be essential constituents of the ancestral tRNA synthetases. The charged residues 225, 230, and 233 are involved in pattern [HKR]-X(4)-[HKR]-X(2)-[HKR] present in several synthetases from class I, located between already known signature HIGH and consensus KMSKS sequences, whereas Asp194 is a part of the GSDQ motif. These residues were possibly conserved due to their catalytic activity for aminoacyl adenylate formation. Such analysis has also confirmed independently the role of already known HIGH and KMSKS signature sequences and identified an additional sequence GSDQ motif and [HKR]-X(4)-[HKR]-X(2)-[HKR] pattern conserved among the class I aminoacyl tRNA synthetases, which further supports the recently proposed classification of RS by Eriani et al. (1990). These results indicate a close relationship between class I aminoacyl tRNA synthetases, suggesting a common evolutionary ancestor. Such hypothetical ancestral enzymes could be characterized by specific three-dimensional structures of the charged residues included in patterns I–IV.

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References

Bajorath J, Kitson DH, Fitzgerald G, Andzelm J, Kraut J, Hagler AT (1991) Electron redistribution on binding of a substrate to an enzyme folate and dihydrofolate reductase. Proteins Struct Funct Genet 9:217–224.

Bhat TN, Blow DM, Brick P, Nyborg J (1982) Tyrosyl-tRNA synthetase forms a mononucleotide-binding fold. J Mol Biol 158:699–709

Breton R, Watson D, Yaguchi M, Lapointe J (1990) Glutamyl-tRNA synthetases of *Bacillus subtilis* 168T and of *Bacillus stearothermopilus*. J Biol Chem 265:18248–18255

Brick P, Bhat TN, Blow DM (1989) Structure of tyrosyl-tRNA synthetase refined at 2.3 Å resolution. Interaction of the enzyme with the tyrosyl adenylate intermediate. J Mol Biol 208: 83–98

Burbaum JJ, Starzyk RM, Schimmel P (1990) Understanding structural relationships in proteins of unsolved three-dimensional structure. Proteins Struct Funct Genet 7:99–111

Carter PJ, Winter G, Wilkinson AJ, Fersht AR (1984) The use of double mutants to detect structural changes in the active site of the tyrosyl-tRNA synthetase (*Bacillus stearothermopilus*). Cell 38:835–840.

de Duve C (1988) The second genetic code. Nature 333:117–118

Eriani G, Delarue M, Poch O, Gangloff J, Moras D (1990) Partition of tRNA synthetases into two classes based on mutually exclusive sets of sequence motifs. Nature 347:203–206

Eriani G, Dirheimer G, Gaugloff J (1991) Cysteinyl-tRNA synthetase determination of the last *E. coli* aminoacyl-tRNA synthetase primary structure. Nucleic Acids Res 19:265–269

Fersht AR (1987) Dissection of the structure and activity of enzyme. Proc R.A. Welch Found Conf Chem Res 31:158–182

Fersht AR, Knill-Jones JW, Bedouelle H, Winter G (1988) Reconstruction by site-directed mutagenesis of the transition state for the activation of tyrosine by the tyrosyl-tRNA synthetase. Biochemistry 27:1581–1587

Garcia GA, Leatherbarrow RJ, Eckstein F, Fersht AR (1990) Metal ion dependence of phosphothioate ATP analogues in the *Bacillus stearothermopilus* tyrosyl-tRNA synthetase reaction. Biochemistry 29:1643–1648

George DG, Barker WC, Hunt LT (1986) The protein identification resource. Nucleic Acids Res 14:11–16

Halgren TA, Lipscomb WN (1977) The synchronous-transit method for determining reaction pathways and locating molecular transition states. Chem Phys Lett 49:225–232

Hountondji C, Dessen P, Blanquet S (1986) Sequence similarities among the family of aminoacyl-tRNA synthetases. Biochimie 68:1071-1078

Hountondji C, Schmitter J-M, Beauvallet C, Blanquet S (1990) Mapping of the active site of *Escherichia coli* methionyl-tRNA synthetase: identification of amino acid residues labeled by periodate-oxydized $tRNA^{fMet}$ molecules having modified lengths at the 3'-acceptor end. Biochemistry 29:8190–8198

Hwang JH, Warshel A (1987) Semiquantitative calculations of catalytic free energies in genetically modified enzymes. Biochemistry 26:2669–2673

Krauss M, Garmer DR (1990) Active site ionicity and the mechanism of carbonic anhydrase. J Am Chem Soc (in press)

Leatherbarrow RJ, Fersht AR (1987) Investigation of transition-state stabilization by residues histidine-45 and threo-

nine-40 in the tyrosyl-tRNA synthetase. Biochemistry 26: 8524–8528

Leatherbarrow RJ, Fersht AR, Winter G (1985) Transition state stabilization on the mechanism of tyrosyl tRNA synthetase revealed by protein engineering. Proc Natl Acad Sci USA 82: 7840–7844

Lowe DM, Fersht AR, Wilkinson AJ, Carter P, Winter G (1985) Probing histidine–substrate interactions in tyrosyl-tRNA synthetase using asparagine and glutamine replacements. Biochemistry 24:5106–5109

Mechulam Y, Dardel F, Le Carre D, Blanquet S, Fayat G (1991) Lysine 335, part of KMSKS sequence plays a crucial role in amino acid activation catalysed by the methionyl-tRNA synthetase from *Escherichia coli*. J Mol Biol 217: 465–475

Meinnel T, Mechulam Y, Dordel F, Schmitter JM, Mountondji C, Brunie S, Dessen P, Fayat G, Blanquet S (1990) Methionyl-tRNA synthetase from *E. coli*—a review. Biochimie 72: 625–632.

Monteilhet C, Blow DM (1978) Binding of tyrosine adenosine triphosphate and analogues to crystalline tyrosyl transfer RNA synthetase. J Mol Biol 122:407-417

Naray-Szabo G (1988) Electrostatic catalysis in enzymes. J Mol Catal 47:281–287

Rein R, Srinivasan S, McDonald J, Raghunathan G, Shibata M (1987) Structural elements and organization of the ancestral translational machinery. Origins Life 17:431–438

Sawaryn A, Sokalski WA (1989) Cumulative atomic multipole moments and point charge models describing molecular charge distribution. Comp Phys Comm 52:397–408

Schimmel P (1989) Parameters for the molecular recognition of transfer RNAs. Biochemistry 28:2747–2759

Schimmel P (1991) Classes of aminoacyl-tRNA synthetases and the establishment of the genetic code. Trends Biochem Sci 16:1–3

Sokalski WA (1985) The physical nature of catalytic activity due to the molecular environment in terms of intermolecular interaction theory: derivation of simplified models. J Mol Catal 30:395-410

Sokalski WA, Maruszewski K, Hariharan PC, Kaufman JJ (1989) Library of cumulative atomic multipole moments: II. Neutral and charged amino acids. Int J Quantum Chem Quantum Biol Symp 16:119–164

Webster TA, Tsai M, Kula M, Mackie G, Schimmel P (1984) Specific sequence homology and three-dimensional structure of an aminoacyl transfer RNA synthetase. Science 226:1315–1317

Wells TNC, Fersht AR (1985) Hydrogen bonding in enzymatic catalysis analyzed by protein engineering. Nature 316:656–657

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