Detection of Noncovalent tRNA:Aminoacyl-tRNA Synthetase Complexes by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry*

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-MS) was used for the study of complexes formed by yeast seryl-tRNA synthetase (SerRS) and tyrosyl-tRNA synthetase (TyrRS) with tRNA<sup>ser</sup> and tRNA<sup>tyr</sup>. Cognate and noncognate complexes were easily distinguished due to a large mass difference between the two tRNAs. Both homodimeric synthetases gave MS spectra indicating intact desorption of dimers. The spectra of synthetase-cognate tRNA mixtures showed peaks of free components and peaks assigned to complexes. Noncognate complexes were also detected. In competition experiments, where both tRNA species were mixed with each enzyme only cognate α<sub>2</sub>γ<sub>2</sub> complexes were observed. Only cognate α<sub>2</sub>γ<sub>2</sub> complexes were detected with each enzyme. These results demonstrate that MALDI-MS can be used successfully for accurate mass and, thus, stoichiometry determination of specific high molecular weight noncovalent protein-nucleic acid complexes.

Translation of the genetic code is the defining characteristic of living systems. The first and most critical step in translation is the highly specific attachment of amino acids to their cognate tRNAs which is catalyzed by aminoacyl-tRNA synthetases (1–3). The synthetases vary in size and subunit composition and can be divided into two classes of 10 enzymes each based on conserved sequences (4) and structural motifs of the active site region (5). These features of individual synthetases are highly conserved in evolution (6). To perform its demanding task, each of the 20 aminoacyl-tRNA synthetases has to discriminate among a collection of tRNAs present in the cell. Molecules of tRNA share a nearly invariant L-shaped tertiary structure which is necessary in the later steps of translation. As indicated by crystal structures of several synthetase-tRNA pairs, members of each synthetase class interact with two different regions of the L-shaped tRNA molecules (7). The formation of both cognate and non-cognate synthetase-tRNA complexes has been observed by a variety of experimental techniques such as nitrocellulose filter binding, fluorescence quenching, equilibrium partition, neutron scattering and polyacrylamide gel electrophoresis, footprinting, stopped-flow etc. (8–14). Cognate tRNAs, however, form a more stable complex with the synthetase and are strongly preferred in aminoacylation. Crystal structures of several cognate synthetase-tRNA complexes recently led to progress in understanding specific recognition between macromolecules (15). The specificity of aminoacylation in vivo is controlled by competition for the substrates (16). Nevertheless, misacylation may occur even in vivo when the ratio between the synthetase and tRNA concentrations is disturbed by overexpression (17).

As a novel approach to gain further insight into the formation and stability of synthetase-tRNA complexes, we have performed a MALDI-MS<sup>1</sup> study of the complexes formed by yeast TyrRS and SerRS with their cognate and noncognate tRNAs. The two yeast aminoacyl-tRNA synthetases are representatives of class I and class II synthetases, respectively. Their amino acid sequences based on gene structures are known (18, 19), and crystal structures of their bacterial counterparts have been determined at high resolution (5, 20, 21). Both yeast enzymes are homodimeric. Based on a gel-shift analysis, yeast TyrRS binds one cognate tRNA molecule per dimer (11). The preferred type of yeast SerRS<sup>γ</sup>-tRNA<sup>ser</sup> complex is probably two tRNAs per synthetase (22, 23). In this paper we provide further evidence regarding the stoichiometry of these complexes. The mass difference between tRNA<sup>tyr</sup> and tRNA<sup>ser</sup> is over 2 kDa due to the long variable arm in tRNA<sup>ser</sup>. This facilitates a ready MS differentiation between cognate and noncognate pairs.

Recently, MALDI-MS has been successfully applied to the detection of a number of intact noncovalent complexes, such as protein quaternary structures (24–28), metal-peptide complexes (27), complementary DNA strands (29), and nonspecific complexes of basic peptides with oligodeoxyribonucleotides (30). Electrospray ionization mass spectrometry has also been proven a general method for the analysis of a variety of noncovalent structures. In particular, the specific noncovalent complex of transcription factor, PU.1 DNA-binding domain with a 17-base pairs double-stranded DNA fragment, has been analyzed, providing an example for the study of a biologically relevant protein-nucleic acid complex (31).

Our present study extends the application of mass spectrometry, in general, and MALDI-MS, in particular, to studies of biologically relevant protein-nucleic acid complexes with

<sup>*</sup>The abbreviations used are: MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; TyrRS, tyrosyl-tRNA synthetase; SerRS, seryl-tRNA synthetase; ATT, 6-aza-2-thiothymine.
masses exceeding $10^4$ Da. High specificity of the recorded interactions between 40–50 kDa-protein and 25–27 kDa nucleic acid constituents is demonstrated.

**MATERIALS AND METHODS**

**Enzymes**—Tyrosyl-tRNA synthetase was prepared from commercial yeast by a modification (32) of published purification steps (33). Seryl-tRNA synthetase was purified from the *Saccharomyces cerevisiae* overproducing strain by a two-step chromatographic procedure on FPLC MonoQ and MonoS columns (Pharmacia Biotech Inc.) as described previously (34). For MALDI-MS experiments, both enzymes were transferred to 0.5 M ammonium acetate, pH 7.0, 5 mM 2-mercaptoethanol using Centricron-10 concentrators (Amicon). Glycerol was then added to a final concentration of 10% and the enzymes were kept at −80 °C.

**Transfer RNA—tRNATyr and tRNAsc** were prepared from yeast tRNA fractions enriched in tyrosine and serine acceptors, kindly supplied by Professor G. Dirheimer (Strasbourg, France). tRNATyr was purified by repeated chromatography of tyrosylated and detyrosylated tRNA on a benzoylated DEAE-cellulose column (35). Purified tRNATyr accepted at least 1.4 nmol of tyrosine/A$_{260}$ unit, determined in an aminocoylation assay described elsewhere (11). To isolate tRNAsc acceptors, the tRNA was serylated using pure seryl-tRNA synthetase, derivatized by naphthoxyacetylation (36), and purified by chromatography on a benzoylated DEAE-cellulose column (37). The aliquots from the chromatography fractions were subjected to electrophoresis on a 8% polyacrylamide gel containing 8% urea. The fractions containing derivatized tRNAAsc, showing the lowest mobility on the gel, were pooled and deacylated. Purified tRNAsc accepted at least 1.2 nmol of serine/A$_{260}$ unit of tRNA. Aminocoylation assays were performed as described elsewhere (34). Both tRNAs were extensively dialyzed against water and kept at −20 °C.

**Preparation of Complexes for MALDI-MS—**Appropriate amounts of tRNAsc or tRNAAsc, as required for individual experiments, were kept at 55 °C for 2 min in 12.5 mM ammonium acetate, pH 7.0, and slowly cooled to 25 °C, thus assuring proper renaturation and conformational integrity before complex formation. Enzyme solutions were added and the mixtures incubated for 5 min at 25 °C, followed by cooling on ice and addition of 12.5 mM ammonium acetate, pH 7.0, to a final glycerol concentration of 6%. Gel retardation electrophoresis was routinely performed on aliquots of all samples subjected to mass spectrometric measurement.

**Gel Retardation Electrophoresis**—100 × 700 × 0.75 mm$^3$ gels were prepared using acrylamide/bisacrylamide (40:1) and 5% glycerol in 12.5 mM ammonium acetate, pH 8.3. Samples in 6% glycerol were loaded on previously cooled gels and electrophoresis was run at 4 °C for 4 h at 5 mA and 50 V using 12.5 mM ammonium acetate, pH 8.3, as the running buffer. The gels were stained with silver (38).

**Mass Spectrometry—**Mass spectra were acquired on a prototype Vison 2000 time-of-flight mass spectrometer (Thermo BioAnalysis, Hemel Hempstead, United Kingdom) modified in our laboratory. It was operated in linear mode with an acceleration potential of 20 kV and delayed ion extraction. All spectra shown were acquired in positive ion mode. Ions were desorbed by irradiation with a frequency-tripled Nd:YAG laser (355 nm, 5 ns; Spectrum GmbH, Berlin, Federal Republic of Germany). The equivalent flight path length was 1.95 m. The base pressure was 10$^{-9}$ Pa. Ions were detected with a discrete dynode secondary electron multiplier (R 2562, Hamamatsu Photonics, Herrsching, FRG) equipped with a conversion dynode for efficient detection of high mass ions. The total impact energy of the ions on the conversion dynode was 39 keV. The preamplified output signal of the secondary electron multiplier was digitized at up to 100 MHz by a transient recorder (LeCroy 9450, LeCroy, Chestnut Ridge, NY) and then transferred to a personal computer equipped with custom-made software (ULISSES, Chip@work, Bonn, FRG). Up to 65 single shot spectra were averaged for the spectra shown.

**Sample Preparation for MALDI-MS—**The MALDI matrix used was 6-aza-2-thiothymine (Sigma). Diammonium citrate (Sigma) or ammonium acetate (Merek) were added to the ATT matrix solution. ATT was dissolved to a concentration of 40 mg in 10 mM diammonium citrate or in 12.5 mM ammonium acetate, pH 7.0 (25, 29). Fresh matrix solutions were prepared daily. The analyte concentration was typically 1.7 μM SerRS or TyrRS and 6.4 μM tRNA. 1 μl of ATT solution and 0.5 μl of analyte solution were mixed on the target and dried in a stream of cold air until small ATT crystals started to form in the glycerol-containing droplet. Thereupon, 1 μl of ATT solution was added to the remaining droplet and the water partly evaporated. Before allowing the preparation to dry down completely, another 1 μl of ATT solution was added.

**RESULTS**

**Formation of Complexes in a Buffer Compatible with MALDI—**To facilitate the analysis of synthetase-tRNA mixtures by MALDI-MS, particular care had to be taken to choose buffer conditions that do not interfere with matrix crystallization. Formation of aminoacyl-tRNA synthetase-tRNA complexes in vitro was generally assumed to require conditions suitable for aminoacylation of tRNA, i.e. the presence of both K$^+$ and Mg$^{2+}$ ions. The presence of alkali and earth alkali cations is well known to adversely affect matrix crystallization and decrease resolution by extensive cation adduct formation, especially in the case of oligonucleotides (39, 40). Therefore, we elaborated conditions for complex formation in volatile salt solutions known to be compatible with MALDI, e.g. ammonium salts which have been described as buffers and MALDI-matrix additives by several authors (25, 29, 41, 42). Our final choice was 12.5 mM ammonium acetate in ultra-pure water, pH 7. The result of an electrophoretic analysis of complex formation under such conditions is shown in Fig. 1.

Both SerRS and TyrRS formed complexes of similar electrostatic mobility with their cognate tRNAs, in agreement with the a$_{2}$tRNA composition (lanes b, d, e, and g). Additionally, TyrRS formed a slower moving noncognate complex with tRNAAsc (lane f). In analogy with the previously detected TyrRS-tRNAAsc complex (11) we ascribe this to an (a$_{2}$b$_{2}$c$_{2}$-tRNA) structure. SerRS and the noncognate tRNAAsc formed a complex with the mobility corresponding to the a$_{2}$tRNA composition (lane c). All bands obtained are poorly resolved as compared with those obtained under standard conditions (11), but, nevertheless, they demonstrate that synthetase-tRNA complexes can be formed in ammonium acetate solution.

**Peak Assignments in MALDI Mass Spectra of Noncovalent Complexes—**The successful analysis of noncovalently bound compounds by MALDI-MS requires that the complex be transferred from the liquid to the solid phase, and incorporated into the matrix crystal. Upon interaction with the laser pulse, intact
complexes must survive the transition from the solid state to the gas phase and be stable in the gas phase on a time scale of some $10^{-4}$ s for detection. Effects known to substantially contribute to noncovalent interactions include hydrophobic and hydrophilic interactions as well as electrostatic and van der Waals interactions. All of these interactions must be assumed to be substantially influenced by the dramatically changing “solvent” environment during both the crystallization and desorption processes.

At any rate, great care has to be taken not to misinterpret or overinterpret the observed signals. For example, it is well known that reactions in the gas phase of the expanding desorption plume may lead to unspecific aggregation of simultane- ously desorbed molecular species present in the sample (43). Characteristically, the signal intensity of these oligomers generated in the gas phase decreases exponentially with an increasing number of constituents as it is rationalized by a lower probability for multiple collisions. In most cases, the stoichiometry of the molecular aggregate giving rise to a given peak can be unambiguously determined from the mass value alone. For a discrimination between the gas phase aggregation and species present as an aggregate in the solid and desorbed as such, it is necessary to also take into account the peak intensity ratios in the spectra. In the following analysis, the peaks observed will be described and special emphasis will be placed on how conclusions concerning their origin can be drawn from the intensity ratios.

The expressions “native” and “intact” used to describe the desorption of a noncovalently bound aggregates do not imply a specific conformation. In fact, all that can be deduced from MALDI mass spectra is the stoichiometry of such aggregates. Actual conformations during crystallization of the matrix and/or the desorption/ionization step need not to be close to the biologically active conformation in cells. However, we will re-

![Image](313x500 to 559x729)

| Substance | Measured mass/Da | Expected mass/Da |
|-----------|------------------|------------------|
| tRNA_Tyr  | 25,325 ± 34a     | 25,348           |
| tRNA_Tyr  | 27,634 ± 50a     | 27,623–27,672b   |
| TyrRS subunit | 42,345 ± 27    | 44,020c         |
| SerRS subunit | 54,413 ± 25    | 53,309d         |

a Determined by external calibration with horse heart apomyoglobin (m/z = 16952 Da) and bovine carbonic anhydrase (m/z = 29023 Da).

b Masses of the three known isoacceptors of tRNA span this range.

c Determined by internal calibration with bovine carbonic anhydrase (m/z = 29023 Da) and bovine serum albumin (m/z = 66431 Da).

d SWISSPROT data base.

![FIG. 2. MALDI-mass spectra of TyrRS and TyrRS-tRNA Tyr complexes. The samples contained 0.86 pmol of TyrRS, 0.12 μmol of ATT, and 0.4 μmol of glycerol. In addition, 38 nmol of ammonium acetate was present in a, and 30 nmol of di ammonium citrate and 3.2 pmol of tRNA Tyr in b. The recordings in a and b show the sums of 2 and 62 single shot spectra, respectively.](313x500 to 559x729)

Figure 2: MALDI-mass spectra of TyrRS and TyrRS-tRNA Tyr complexes. The samples contained 0.86 pmol of TyrRS, 0.12 μmol of ATT, and 0.4 μmol of glycerol. In addition, 38 nmol of ammonium acetate was present in a, and 30 nmol of di ammonium citrate and 3.2 pmol of tRNA Tyr in b. The recordings in a and b show the sums of 2 and 62 single shot spectra, respectively.

The peak intensity ratio for this kind of spectrum indicates intact desorption of the TyrRS dimer, as the dimer peak is the base peak and the corresponding to 4 α-subunits is higher than that corresponding to 3 α-subunits. If the homoligomeric signals of α-subunits originated from unspeci- fic gas phase association only, their intensity would decrease exponentially with an increasing number of constituents. However, minor contribution of the latter effect cannot be excluded. This peak intensity ratio was only observed for the first laser shot at a given sample location.

From the second shot on, the monomer was the base peak of the spectrum with the peak intensity decreasing with increasing mass. This behavior was previously observed for a variety of protein quaternary structures (24, 26, 43).

To demonstrate the ability to detect synthetase-tRNA complexes by MALDI-MS a sample of TyrRS was mixed in a vial with an excess of tRNA Tyr (see “Materials and Methods”) prior to deposition on the target and addition of the matrix (Fig. 2b). In the mass spectrum the two most prominent peaks could be assigned to tRNA Tyr and the protein α₁-subunit (cf. Table I). An additional peak at m/z = 50650 corresponds to the m/z value of 2 tRNA Tyr molecules which, in all likelihood, reflects an unspecific gas phase aggregation. The protein dimer signal is not present in this spectrum. Instead, two signals at m/z = 110,078 Da and m/z = 135,541 Da have appeared, corresponding to a stoichiometry of 2 α-subunits of TyrRS plus one and two tRNA Tyr molecules, respectively. They indicate intact desorption of the synthetase-tRNA complex. If the signal had been due to unspecific gas phase association the intensity ratios could not be explained satisfactorily because of the missing peak of the TyrRS dimer. The presence of the signal corre-
Most abundant. SerRS, mixed with an excess of tRNASer, gave a dimer peak, a signal attributed to $\alpha_2$-tRNA$_{\text{ser}}$ complex. Essentially the same peak intensity distribution as in the mass spectrum of the enzyme as a native dimer (base peak).

The origin of molecular ion signals assigned to complexes—

Origin of Molecular Ion Signals Assigned to Complexes—To investigate the specificity of binding between tRNA and the synthetases, the correlation between spectral appearance and the concentrations in solution, as well as to discriminate against gas phase phenomena, further experiments were performed.

In addition to the usual synthetase/tRNA ratios (Figs. 4a and 5a, cf. also Figs. 2b and 3b), mass spectra of mixtures with an excess of the enzyme over cognate tRNA were acquired (Figs. 4b and 5b). In these spectra the ratio of the protein dimer peak intensity to the $\alpha_2$-tRNA complex peak intensity increases. Peaks corresponding to the $\alpha$-subunits, tRNA, and $\alpha_1$-tRNA are present. In the SerRS case, peaks at $\tilde{m}/\tilde{z}$ = 163 and $\sim$220 kDa corresponding to 3 and 4 $\alpha$-subunits of the protein (Fig. 5b) were also observed. The peak intensity ratio of the latter two indicates gas phase association of two intacty desorbed SerRS dimers. The formation of $\alpha_2$-tRNA$_{\text{cyt}}$ complexes (11) could neither be excluded nor confirmed. Spectra of noncognate synthetase/tRNA mixture are shown in Figs. 4c and 5c. In addition to the signals of tRNA and enzyme subunits, both spectra show protein dimer and noncognate $\alpha_1$-tRNA complex signals. In the SerRS case, a peak corresponding to $\alpha_1$-tRNA$_{\text{ser}}$ was observed, as was the case with cognate tRNA. The expected and experimentally determined mass values for the

The amounts of material per sample were: 0.86 pmol of TyrRS, 3.2 pmol of tRNA$_{\text{ser}}$, 0.12 pmol of ATT, 0.4 $\mu$mol of glycerol and 30 nmol of diaminomycin citrate. The recordings were applied to the target separately (see text) and 38 nmol of ammonium acetate was used instead of citrate. The recordings $a$, $b$, $c$, and $d$ show the sums of 62, 28, 10, and 6 single shot spectra, respectively. Spectrum $a$ is identical to that shown in Fig. 2b and repeated here for ease of comparison.

FIG. 4. MALDI-mass spectra of TyrRS and its complexes with cognate and noncognate tRNA. Except when stated otherwise, the amounts of material were the same as in $a$, but the components were applied to the target separately (see text) and 38 nmol of ammonium acetate was used instead of citrate. The recordings $a$, $b$, $c$, and $d$ show the sums of 62, 28, 10, and 6 single shot spectra, respectively. Spectrum $a$ is identical to that shown in Fig. 2b and repeated here for ease of comparison.

The spectra of analogous experiments performed with SerRS and tRNA$_{\text{ser}}$ are shown in Fig. 3. Spectrum $a$ represents a mass spectrum of the enzyme as a native dimer (base peak). Essentially the same peak intensity distribution as in the TyrRS case is observed because the protein dimer ion is the most abundant. SerRS, mixed with an excess of tRNA$_{\text{ser}}$, gave the spectrum shown in Fig. 3b. In addition to the native SerRS dimer peak, a signal attributed to $\alpha_2$-tRNA$_{\text{ser}}$ ($m/z$ = 136,144 Da) was also observed. Another weak and poorly resolved signal at $m/z$ = 162,560 Da could not unequivocally be attributed to either the $m/z$ value of 3 $\alpha$-subunits of SerRS or an $\alpha_2$-tRNA$_{\text{cyt}}$ complex. The same holds true for a signal at $m/z$ = 64 $\approx$ 82 kDa, where the signal to noise ratio and resolution of the signal did not allow assignment to either 3 tRNA$_{\text{ser}}$ or $\alpha_1$-tRNA$_{\text{ser}}$. In contrast to the TyrRS case, the protein dimer ion signal intensity is comparable to that of the $\alpha_2$-tRNA$_{\text{ser}}$ signal. The peak intensity distribution of the spectrum indicates that the signals denoted $\alpha_2$ and $\alpha_2$-$\gamma$-tRNA$_{\text{ser}}$ reflect species desorbed intacty rather than gas phase aggregates since the intensity of each peak is higher than that for the 3 tRNA$_{\text{ser}}$/$\alpha_1$-tRNA$_{\text{cyt}}$ peak. In the case of SerRS, the “first shot behavior” described above was also observed while it was not for its $\alpha_2$-tRNA$_{\text{ser}}$ complex.

Experiments for Figs. 2 and 3 were carried out with either ammonium acetate or diammonium citrate as additives to the ATT solution, with essentially equal results. The typical mass resolution obtained for protein dimer signals and $\alpha_2$-tRNA complexes was $m/dm = 30$ (full width at half-maximum).

FIG. 3. MALDI-mass spectra of SerRS and SerRS-tRNA$_{\text{ser}}$ complexes. The samples contained 0.86 pmol of SerRS, 0.12 $\mu$mol of ATT, and 0.4 $\mu$mol of glycerol. In addition, 30 nmol of diaminomycin citrate was present in $a$, and 38 nmol of ammonium acetate and 3.2 pmol of tRNA$_{\text{ser}}$ in $b$. The recordings in $a$ and $b$ show the sums of 11 and 16 single shot spectra, respectively.

Experiments for Figs. 2 and 3 were carried out with either ammonium acetate or diammonium citrate as additives to the ATT solution, with essentially equal results. The typical mass resolution obtained for protein dimer signals and $\alpha_2$-tRNA complexes was $m/dm = 30$ (full width at half-maximum).

Origin of Molecular Ion Signals Assigned to Complexes—To investigate the specificity of binding between tRNA and the synthetases, the correlation between spectral appearance and...
Table II

| Peak assignment | Measured mass/Da | Expected mass/Da | Δm/Da |
|-----------------|------------------|------------------|-------|
| (TyrRS dimer)·tRNA<sub>Tyr</sub> | 110,078 | 110,015 | +63 |
| (TyrRS dimer)·2 tRNA<sub>Tyr</sub> | 135,541 | 135,340 | +201 |
| (SerRS dimer)·tRNA<sub>Ser</sub> | 136,144 | 136,460 | -316 |
| SerRS trimer | 162,590 | 163,239 | -649 |
| (SerRS dimer)·2 tRNA<sub>Ser</sub> | 162,590* | 164,094 | -1504 |
| (TyrRS dimer)·tRNA<sub>Ser</sub> | 112,053 | 112,324 | -271 |
| (SerRS dimer)·tRNA<sub>Tyr</sub> | 133,913 | 134,151 | -238 |

* Unequivocal peak assignment not possible with the achieved mass resolution.

Fig. 5. MALDI-mass spectra of SerRS and its complexes with cognate and noncognate tRNA. Except when stated otherwise, the amounts of material per sample were: 0.86 pmol of SerRS, 3.2 pmol of tRNA<sub>Ser</sub>, 0.12 μmol of ATT, 0.4 μmol of glycerol, and 38 nmol of ammonium acetate (a). In b an excess of SerRS (3.1 pmol) over tRNA<sub>Ser</sub> (1.6 pmol) was used. In c, 3.2 pmol of tRNA<sub>Tyr</sub> was used instead of tRNA<sub>Ser</sub>. In d the amounts of material are the same as in a, but the components were applied to the target separately (see text). The recordings a, b, c, and d show the sums of 16, 16, 18, 20, and 6 single shot spectra, respectively. Spectrum a is identical to that shown in Fig. 3b and repeated here for ease of comparison.

Complexes detected are given in Table II.

To prove that the signal at the m/z value of the α<sub>2</sub>·tRNA complexes is not a gas phase artifact, we tried to achieve a situation in which tRNA and synthetase are both present in the spectra with comparable signal intensities without previously allowing them to form a complex. For this purpose, we prepared layered samples containing pure SerRS or TyrRS solutions and processed them as described before. On the target, only after the last drying step, 0.5 μl of cognate tRNA solution was added and dried down. The formation of a complex between synthetase and tRNA can be assumed not to occur under these conditions. In the single laser shot spectra obtained from this preparation, most spots yielded only synthetase or only tRNA. For Figs. 4d and 6d, however, only single shot spectra from spots showing both the synthetase and the tRNA with comparable signal intensities were summed up. No peaks corresponding to protein/nucleic acid aggregates were observed. A signal corresponding to the mass value of the α<sub>2</sub>·tRNA complexes at m/z = 110,015 Da (Tyr) and m/z = 136,460 Da (Ser) was missing in each case. In addition, the peak intensity distribution for both tRNA and synthetase shows the exponential decrease of peak area with an increasing number of constituents typical for gas phase association. However, the presence of both tRNA<sub>Ser</sub> and SerRS in a single shot spectrum in comparable intensities is not unambiguous due to the overlap of the tRNA<sub>Ser</sub> signal with the doubly charged SerRS subunit α<sub>2</sub><sup>+</sup> ion signal.

Competition of tRNAs for Synthetase Binding—All the results shown in Figs. 2–5 still leave some doubt as to whether the peaks corresponding to α<sub>2</sub>·tRNA are really due to specific recognition of cognate tRNA by the synthetases in solution. Artifacts generated during crystallization of the matrix or in the gas phase after desorption could not definitely be ruled out. To prove the attribution of these ion signals to complexes formed in the solution state, experiments with mixtures containing equimolar concentrations of both cognate and noncognate tRNAs which compete for binding to the α<sub>2</sub> protein were performed for each synthetase.

In Figs. 6 and 7 the mass spectra obtained from these solutions are shown. Results obtained with both enzymes show the same regularity in the formation of complexes. Peaks corresponding to the enzyme α<sub>2</sub>-subunit, the enzyme α<sub>1</sub>-dimer, both tRNAs and α<sub>2</sub>·tRNA were detected. Among the α<sub>2</sub>·tRNA and α<sub>2</sub>·tRNA<sub>2</sub> complexes only signals with a centroid mass value corresponding to cognate synthetase·tRNA complexes were detected.

Because of the limited mass resolution achievable in this mass range with the given sample, minor contributions of components with a slightly different molecular mass cannot be distinguished. However, it should be noted that all mass values were determined by centroiding the respective peak without additional assumptions about its shape.

All measured and expected mass values for this experiment are shown in Table III. Because of the relatively low mass resolution, minor contributions of components with a slightly different molecular mass cannot be distinguished. However, it should be noted that all mass values were determined by centroiding the respective peak without additional assumptions about its shape. It is these measured centroid values that are given in Table III. A comparison of the deviations Δm of these values from the expected molecular masses (cf. last column of Table III) with the 2.3-kDa mass difference between tRNA<sub>Tyr</sub> and tRNA<sub>Ser</sub> clearly demonstrates the predominance of cognate over noncognate complex signals. To further illustrate this point, the position of the centroids of the respective noncognate complexes (in parentheses) is shown in the insets in Figs. 6 and 7. For the SerRS case, the expected mass of α<sub>2</sub>·tRNA<sub>Ser</sub> is very similar to the mass value of 3 subunits of SerRS, rendering an unequivocal differentiation between 3α and α<sub>2</sub>·tRNA<sub>Ser</sub> difficult.

It is known that in solution the dissociation constant of cognate synthetase·tRNA complexes is about an order of magnitude lower than that of their noncognate counterparts. In principle, both types of complexes can be formed as is evidenced by earlier work (11) and by our present measurements (cf. Figs. 6 and 7).
The amount of material per sample was: 0.86 pmol of TyrRS, 3.2 pmol of tRNATyr, 3.2 pmol of tRNASer, 0.12 μmol of ATT, 38 nmol of ammonium acetate, and 0.4 μmol of glycerol. Sum of 32 single shot spectra is recorded.

1c, 1f, 4c, and 5c). Because of the order of magnitude difference in their dissociation constants, in solution cognate complexes should be formed in a very high excess if both tRNAs are present in equimolar concentrations.

Therefore, the predominant detection of cognate complexes in our mass spectra despite the equimolar presence of both cognate and noncognate tRNA in solution and during crystallization and desorption/ionization clearly shows that possible artifacts introduced by the MALDI preparation and detection process do not contribute appreciably to the α₂tRNA₂ complex signals.

**DISCUSSION**

*What Is the Fate of Noncovalent Complexes during MALDI Sample Preparation?*—It has to be assumed that noncovalent complexes will be substantially influenced during preparation of the MALDI sample, when solid crystals containing both matrix and analyte form. On the one hand, in MALDI mass spectra, oligomer signals corresponding stoichiometrically to the protein’s quaternary structure appear as the base peak in the spectrum only for the first laser shot on a new sample location. For all consecutive laser shots, monomers dominate the spectra. This phenomenon was previously observed by our group (24, 26, 43) and confirmed in this study for the aminoacyl-tRNA synthetases. However, the tRNA-synthetase complexes appear as stoichiometrically intact noncovalent structures for many consecutive laser shots. In this context, the role of glycerol has to be discussed. Glycerol is present in high molar excess over both matrix and analytes in the samples analyzed in this study, which is very unusual for UV-MALDI preparations. The observation that many consecutive single shot spectra which showed the protein-nucleic acid complex could be obtained from one sample location could have been explained under the assumption that the complexes remain dissolved in the glycerol droplet. If the glycerol provided a “self-healing” sample surface, in between two laser shots new liquid containing the complex could replace the desorbed material. However, for the synthetase dimer the first shot phenomenon was observed. This cannot be explained satisfactorily if desorption out of the liquid glycerol played an appreciable role as in these preparations the same amount of glycerol is present. Thus, it must be assumed that the protein-nucleic acid complex is incorporated into the matrix crystal while the homodimer of the enzyme is not.

Recognition of tRNAs by their cognate aminoacyl-tRNA synthetases is usually ascribed to the specific hydrogen bonding of amino acids in the enzyme to the sugar-phosphate backbone and bases in tRNA (44–46), although ionic forces are involved in general synthetase-tRNA interactions. Following Coulomb’s law, these electrostatic forces will increase as the dielectric constant of the environment is lowered by about an order of magnitude during solvent evaporation, thus offering a possible explanation why the synthetase-tRNA complexes remain stable during incorporation into the matrix crystal lattice. It could be assumed that specific recognition is dominated by solution behavior, where effects other than Coulomb attraction contribute appreciably and even dominate recognition. Upon solvent evaporation, the role of these effects diminishes and electrostatic forces become more important, thus stabilizing aggregates whose formation was originally directed by other mechanisms.

In the case of protein quaternary structure formation, electrostatic forces do not contribute to such an extent. Thus, the synthetase dimers readily form in solution but are dissociated upon incorporation into the crystal. Only in the very outermost layers of the crystal can the protein quaternary structures remain in a conformation stable enough to be desorbed as complexes. This proposed reasoning is at the moment only a working hypothesis based on conjectures and will require further experiments to be validated.

*How Can Misinterpretation of Gas Phase Aggregation of Molecular Ions be Excluded?*—Unspecific formation of both homodimer and heterodimers of compounds present in the sample is known to occur in the MALDI desorption plume to a considerable extent (43). Signals assigned to intactly desorbed oligomers obviously show at the same m/z values as the gas phase aggregates of separately desorbed subunits. The only characteristic feature of oligomers generated in the gas phase is their exponentially decreasing signal intensity with an in-

**TABLE III**

| Peak assignment | Measured mass/Da | Expected mass/Da | Δm/Da |
|-----------------|-----------------|-----------------|-------|
| (TyrRS dimer) tRNATyr | 110,154 | 110,015 | +139 |
| (SerRS dimer) tRNASer | 136,660 | 136,460 | +200 |
| (TyrRS dimer) · 2 tRNATyr | 136,015 | 135,340 | +675 |
| SerRS trimer | 163,444 | 162,539 | +205 |
| (SerRS dimer) · 2 tRNASer | 163,444* | 164,094 | −650 |

*a Unequivocal peak assignment not possible with the achieved mass resolution.
creasing number of constituents. In our study, this pattern appeared when we deliberately avoided complex formation in solution by preparing layered samples and thus let only the gas phase aggregation dominate spectral appearance (see “Results”). The resulting spectra are shown in Figs. 4d and 5d. This is the reason why, in the present study, we hesitate to ascribe the α_{tRNA} signals observed a correlation to species existing in solution. Even though their existence in solution cannot be excluded judging from the spectra, the peak intensity ratios for this kind of aggregates are too similar to that of gas-phase complexes to offer a basis for the assumption that they are formed in solution already.

For both the synthetase dimers and the synthetase-tRNA complexes, however, the corresponding signals are substantially more intense than signals with a lower number of constituents which, in fact, the spectra often lack. If the complex signals were due to unspecific gas phase adduct formation it would be difficult to explain why aggregates of 3d components (α_{tRNA}) appear at higher signal intensity than the α_{dimer}, since the probability of ternary as compared with binary collisions is evidently lower. We exclusively assigned signals to solution state complexes that showed a peak intensity ratio which could be rationalized by intactly desorbed species in this way.

On the other hand, the known gas phase aggregation can help in assigning peaks to intactly desorbed species. For both synthetases, the peak corresponding to 4a-subunits is higher than that corresponding to 3a subunits. This can be rationalized assuming intactly desorbed dimers which, in the MALDI desorption plume, form unspecific binary aggregates. In this picture, the signal corresponding to 3a represents a gas phase trimer of α-monomers or a doubly charged ternary complex of dimers (3α_{tRNA})^{2+}.

Taking the precautions against misinterpretation outlined above, we ascribe the protein dimer as well as the α_{tRNA} and α_{tRNA} signals detected to complexes formed in solution based on molecular recognition rather than in unspecific collisions in the MALDI plume. However, minor contribution by the latter effect, inherent to the MALDI process cannot be ruled out.

How Is the Specificity of Macromolecular Interaction Influenced by the Concentration and the Ratio of Interacting Components?—The aminoclaylation of a particular tRNA is the net result of two types of interaction, a productive interaction with the cognate tRNA synthetase and nonproductive interactions with each of the 19 noncognate synthetases (47). Since tRNAs are not present in substantially higher concentrations than their cognate synthetases (48) and the accurate translation is also strongly influenced by the physiological substrate concentrations which are often below the K_{m} values of the appropriate enzymes (49), we kept the concentrations of the two macromolecular partners as well as their ratio during complex formation at the level which corresponds to the situation in vivo.

The Stoichiometry of Complexes between Aminoacyl-tRNA Synthetases and Their Cognate tRNAs—The most important contribution of mass spectrometry toward understanding the recognition of tRNAs by the aminoclayl-tRNA synthetases is the ready and unambiguous determination of the stoichiometry of their complexes. Class I synthetases are predominantly monomers, except TrpRS and TyrRS, whereas class II synthetases are obligate homodimers or heterodimers (3). Interestingly, dimeric enzymes may bind one or two molecules of tRNA. In some systems each molecule of cognate tRNA interacts with only one enzyme subunit while in others cross-subunit binding of tRNA occurs. Thus, not only is the dimeric nature of the enzymes essential for correct conformation of the active site but intersubunit communication may also promote substrate specificity (15). Apart from a very few exceptions (50), the oligomeric structure of aminoacyl-tRNA synthetases specific for particular amino acids is conserved in evolution while this does not seem to be the case for the number of tRNAs simultaneously bound per dimer. The most studied tyrosyl-tRNA synthetase isolated from *Bacillus stearothermophilus* binds one tRNA across two subunits (51). A similar type of complex was detected between *Thermus thermophilus* SerRS and its cognate tRNA (46) while two molecules of tRNA^{Ser} interact with dimeric SerRS from *Escherichia coli* (46, 52). The existence of two types of complexes formed by yeast tyrosyl-tRNA synthetase with cognate tRNA was detected previously by gel retardation assay (11). The composition of the complex formed at ratios of tRNA^{Tyr}/enzyme lower than 0.5 was assigned to be α_{tRNA} in that study while the structure of the complex formed at saturating concentration of tRNA^{Tyr} was ascribed to the α_{dimer} type. In the present work, only the latter was detected in MALDI mass spectra which, in addition, show peaks at m/z values corresponding to two tRNAs interacting with the dimer. Since the specific α_{tRNA} complex signal is also detected in the competition experiment in the presence of noncognate tRNA^{Ser}, we believe this complex is formed in solution and does not represent a gas phase adduct. Therefore, the MS experiments suggest that yeast TyrRS besides the most preferred complex α_{tRNA} may also form symmetrical complexes which cannot be detected by gel retardation assay due to their lower stability. The same stoichiometry of the complexes detected for the serine system is consistent with biochemical experiments indicating the binding of two molecules of tRNA to the dimeric enzyme with different affinities (53).

As we have shown in this study, MALDI-MS provides a ready method for determining the stoichiometry of protein-nucleic acid heterocomplexes. In contrast to the established gel electrophoretic methods that detect mobility, here mass is determined and, thus, stoichiometry can be deduced directly without resorting to assumptions on the correlation between mobility in the electric field and composition of a given complex. Furthermore, a MALDI mass spectrum is acquired in minutes, while a gel electrophoretic analysis with subsequent staining of the gel requires several hours. There are, however, problems involved in the interpretation of MALDI mass spectra of noncovalently bound compounds. Artifacts inherent to the MALDI process may be superimposed on complex signals resulting from specific macromolecular recognition. These problems can be overcome by choosing suitable control experiments and taking precautions against overinterpretation, as demonstrated in this study. In this article, we have demonstrated that MALDI-MS lends itself as a useful and possibly general tool for the study of noncovalent macromolecular interactions.

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