Hg-Coordination Studies of Cysteine Containing Oligopeptides *

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In order to study the interaction of cysteine containing peptide chains with Hg(II) the nuclear quadrupole interaction (NQI) of ^{199m}Hg in the Hg complex of the oligopeptide **A**lanine-**A**lanine-**C**ysteine-**A**lanine-**A**lanine (AACAA) was determined by time differential perturbed angular correlation. Different ^{199m}Hg–NQI's for free and resin-bound AACAA were obtained. Furthermore, the ^{199m}Hg–NQI's are influenced by the Hg:AACAA stoichiometry.

Key words: TDPAC, Nuclear quadrupole interaction, Oligopeptides, Hg-Coordination.

1. Introduction

Although mercury-thiolate chemistry is dominated by an almost linear two-fold Hg coordination, e.g. Hg(cysteine)$_2$ [1] and Hg-ferrocenethiols [2], studies of Hg(II)-biopolymer complexes including Hg-substituted blue copper proteins [3] and the Hg(II) biosensor MerR protein have revealed higher coordination numbers for Hg(II). In the MerR protein, the Hg(II) is threefold coordinated by the thiol groups of three cysteines [4–6] and in tert-butyl-mercaptide, a fourfold Hg-S-coordination is found [1]. In recent years interest has focused on the bio-thio-chemistry of Hg(II), with emphasis on organomercury and aminoacid-mercury compounds, especially with cysteines, stimulated by the ultrasensitivity and extreme selectivity of the MerR protein [4, 5].

In the past we have established that different Hg-S coordinations are easy to differentiate by the nuclear quadrupole interaction (NQI) of ^{199m}Hg monitored by time differential perturbed angular correlation (TDPAC) [1–3, 7, 8].

Here, we report on the interaction of Hg(II) with **AACAA**-oligopeptides, a linear peptide chain with the sequence **a**lanine, **a**lanine, **c**ysteine, **a**lanine, **a**lanine, studied by ^{199m}Hg-TDPAC. Whereas cysteine has a strong affinity to Hg(II) due to its thiol group, alanine displays no propensity to bind metals. To estimate the influence of the Hg(II) concentration and of spatial constraints on the coordination geometry of Hg(II), we performed TDPAC experiments with different Hg:AACAA stoichiometries and with free and resin-bound AACAA. The latter is an AACAA-oligopeptide linked with its C-terminal carboxyl group to a polystyrol matrix via polyoxyethylene spacers.

2. Experimental Methods

The free AACAA oligopeptide was synthesized on an Applied Biosystems 430 A (Weiterstadt, Germany) peptide synthesizer (0.25 mmol scale) with on-line monitoring of the Fmoc-deprotection step [9]. Alanine was attached manually to HMP resin (1 mmol/g) using N,N-dimethylaminopyridine. The loading capacity was 0.55 mmol/g, determined by a piperidine-fulvene assay. The thiol group of cysteine was protected by a triphenylmethyl-group. The aminoacid derivatives were activated by HBTU/HOBt in DMF. The peptide was cleaved from the resin and deprotected by treatment with 12.5% reagent K (thioanisol:phenol:ethandithiol = 2:2:1) and 5% water in TFA at room temperature for 2 h. The resin was filtered off and the peptide was precipitated by diethyl ether/hexane (1:1; V:V) at −25 °C. After centrifugation the pellet was washed twice with diethyl ether/hexane (1:1; V:V) and dissolved in water. After lyophilization the peptide was purified by RP-HPLC on a Waters 600 multisolvent delivery system (Eschborn, Germany) using a

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Eurochrom C18 20 nm 10 µm-column (16 mm · 150 mm). After isocratic elution with 0.1% aq. TFA for 4 min, the peptide was eluted with a linear gradient to 40% aq. acetonitrile (0.1% TFA) in 40 min. The flow rate was 6 ml/min at room temperature. The fractions were detected at 220 nm. After lyophilization, the peptide fraction was stored at −25 °C. The sequence was confirmed by peptide sequencing on an Applied Biosystems 473 A with standard norm cycles.

Furthermore we synthesized the same peptide on a TentaGel S Amin-resin (Rapp Polymere, Tübingen, Germany) using the described chemistry and cleavage conditions. Because of the acid stable peptide-resin linkage, the peptide was deprotected but not cleaved from the resin by TFA.

^{199m}Hg from the radioactive beam at the isotope separator ISOLDE/CERN was implanted into ice (300 µl of Milli-Q plus water). In this way, we obtained essentially carrier-free ^{199m}Hg activity. The main contamination was ^{199g}Hg which we estimate to be a factor of 100–1000 greater in concentrations than ^{199m}Hg. The ^{199m}Hg-implanted ice was thawed and evaporated to 100 µl in order to guarantee a small sample volume. To allow stoichiometric preparations in the range of the oligopeptide concentration, cold $HgCl_2$ was added as carrier to the ^{199m}Hg-solution.

Then the 100 µl of the freshly-prepared solution of the free or resin-bound oligopeptide AACAA (0.1 M TRIS buffer, $pH = 7.2$, oligopeptide concentration 10 mg/ml) was incubated for 2–5 minutes with the ^{199m}Hg-solution with and without carrier. Preparing the Hg(II)-AACAA complex with carrier-free activity only may be called the "infinite dilution" method. After the incubation, the sample was frozen in liquid nitrogen.

The TDPAC measurements were performed at 77 K. Since the half-life of ^{199m}Hg is 43 minutes only and the observed NQI's were rather high, we had to use the highly efficient TDPAC-Camera [10] equipped with BaF_2-scintillators. The time resolution was about 650 ps FWHM.

Used abbreviations:

Fmoc	fluorenylmethyloxycarbonyl
DMF	N,N-dimethylformamide
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
RP-HPLC	reversed-phase high-performance liquid chromatography
TFA	trifluoroacetic acid
HMP	*p*-alkoxybenzylalcohol

3. Results

In Fig. 1 the TDPAC time spectra (left) and their Fourier transforms (right) of the Hg(II)-complexes with free and resin-bound AACAA in frozen solution at 77 K with different stoichiometries are shown. The time spectra were analyzed using a least squares fitting routine allowing for a Lorentzian line broadening; derived NQI parameters are listed in Table 1.

3.1. Free AACAA-Oligopeptide

The ^{199m}Hg-TDPAC spectra of the complex Hg(II)/free AACAA at "infinite dilution" and at a stoichiometry with 10 times less Hg(II) than AACAA exhibit quite a high NQI-frequency $\nu_Q = 1.52(1)$ GHz and a small asymmetry parameter $\eta = 0.19(2)$. This is quite similar to the NQI of Hg(cysteine)$_2$ ($\nu_Q = 1.41(2)$ GHz, $\eta = 0.16(1)$) [2]. Therefore, only a two fold Hg-coordination is formed in the Hg-AACAA-complex.

At the stoichiometric condition Hg:AACAA = 1:2, only a broad frequency distribution centered around 0.72(2) GHz is observed. This significantly lower NQI-frequency indicates a higher coordination number of Hg. Compared to other model compounds, a strongly distorted fourfold coordination seems to be most probable [3]. Furthermore, the rather high degree of line broadening indicates the presence of a multitude of higher coordinated binding sites for mercury. A possible explanation for the absence of a significant contribution of twofold coordinated Hg is that the cysteine thiol groups of the oligopeptides tend to form disulfide bridges with each other. Another reason might be that the deprotection of the cysteine thiol groups during the synthesis of the oligopeptides

Table 1. The hyperfine parameters of free and resin-bound AACAA at different concentrations (1:∞ means "infinite dilution").

Free AACAA:				
Hg : AACAA	ν_Q [MHz]	η	δ [%]	
1 : ∞	1522 (8)	0.19(2)	6.8(7)	
1 : 10	1524 (6)	0.19(1)	3.3(4)	
1 : 2	719(16)	0.61(4)	20 (3)	
Resin bound AACAA:				
Hg : AACAA	ν_Q [MHz]	η	δ [%]	frac. [%]
1 : ∞	637(31)	0	15(9)	21(8)
	1446(21)	0.16(3)	9(6)	79(5)

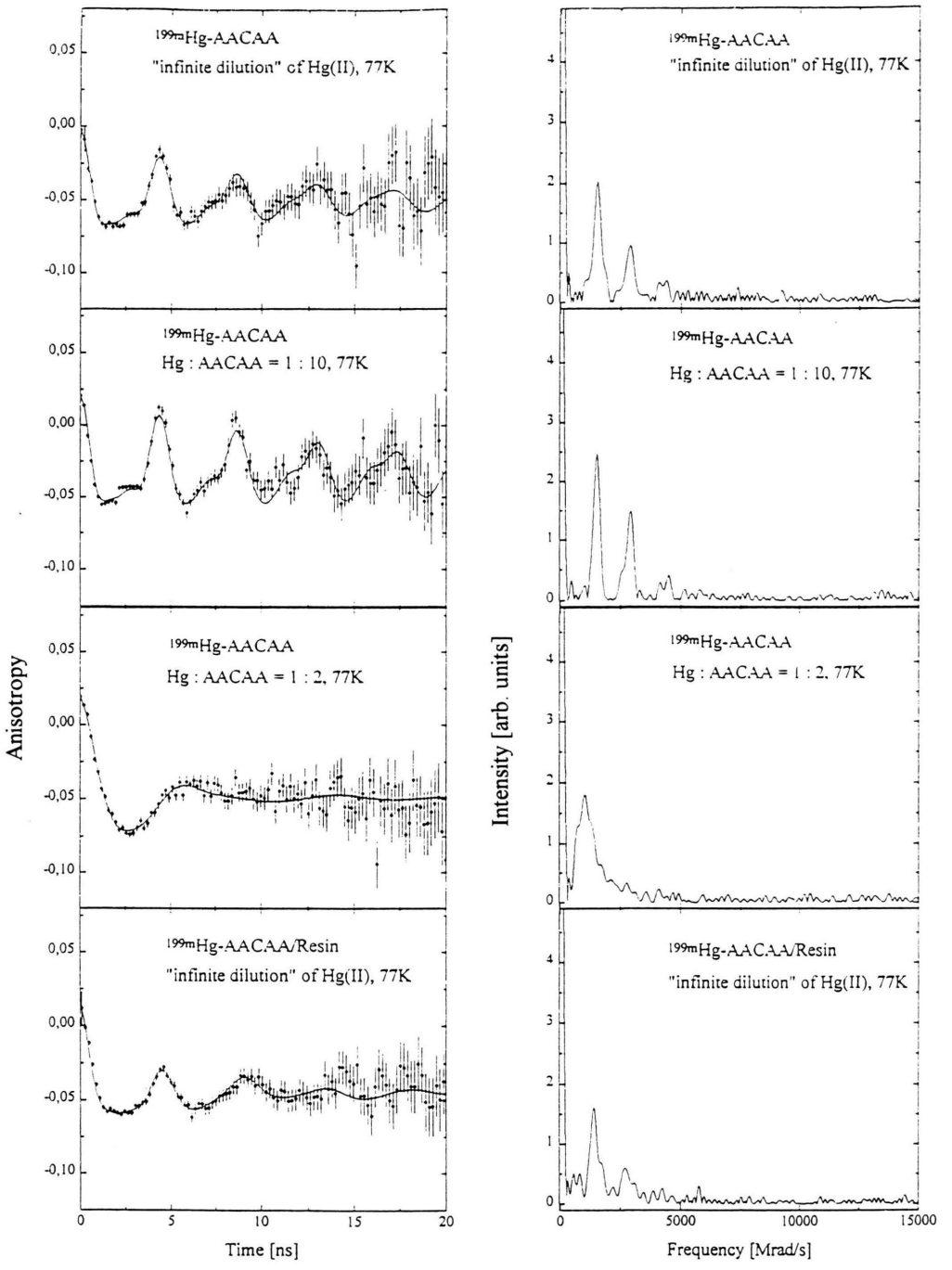

Fig. 1. TDPAC-time spectra (left) and their Fourier transforms (right) of the Hg(II)-AACAA complexes with free and resin bound AACAA in frozen solutions at 77 K with different concentrations. The conversion from the precession frequency ω (and η) into $\nu_Q = e Q V_{zz}/h$ is described in [11].

does not work as quantitatively as generally thought. Both effects result in a surplus of Hg(II) compared to the available cysteine thiol groups, which may lead to higher Hg(II) coordination numbers in the Hg(II)/AACAA complexes.

The spectrum measured at a Hg:AACAA-concentration of 1:10 shows a much smaller line broadening. However, the line broadening in the case of "infinite dilution" is again higher than at the 1:10 Hg-AACAA stoichiometry. It has to be noted that any deviation of the S-Hg-S-bond angle of 180° will lead to a non-vanishing asymmetry parameter η. The observed line broadening and the observed asymmetry parameters are therefore in agreement with the assumption of a distribution of S-Hg-S-angles around 180° caused by the influence of free cysteine thiol groups of other AACAA oligopeptides. Consequently, the different line broadening in the infinite dilution of Hg and the 1:10 Hg/AACAA concentration can be related to the different amounts of free cysteine thiol groups at the 1:10 Hg-AACAA stoichiometry.

From our previous studies with Hg(II)-complexes, the freezing of the complex solution usually does not lead to an appreciable line broadening compared to the instrumental resolution [1, 2].

3.2. Resin-Bound AACAA-Oligopeptide

Here, only the case of "infinite dilution" of Hg was investigated. The TDPAC spectra show two different NQI's with line broadening: the minority fraction with a lower NQI indicates again Hg(II) sites with higher coordination numbers, whereas the majority fraction has almost the same NQI parameters as the free Hg-AACAA-complex prepared under the same conditions. Obviously, the formation of a linear S-Hg-S coordination is hampered by the fixation of the oligopeptide. As far as the metal coordination chemistry is concerned, the resin-bound AACAA-oligopeptides do not behave like free AACAA-oligopeptides. Here, the statement that the polyoxyethylene spacers with a typical length of about 68 ethylene oxide units provide the AACAA-oligopeptides with the full mobility as in liquid phase [12] is only partly valid.

In conclusion, these experiments clearly show that at "infinite dilution" of Hg(II) and free AACAA-oligopeptides only twofold, mainly linear Hg-coordinations are formed. Higher coordination numbers appear if AACAA-oligopeptides are overloaded with Hg(II) or due to spatial constraints, e.g., the fixation on a resin. Therefore, the unusual threefold coordination of Hg(II) at "infinite dilution" by cysteine thiol groups [6] in the MerR protein is undoubtedly forced by the characteristic rigid peptide conformation of the protein.

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