Eur Biophys J (1991) 20:193–201

European Biophysics Journal

Rotational correlation times of peptides determined by perturbed angular correlations of γ-rays

E. Danielsen [1], R. Bauer [1], and D. Schneider [2]

[1] Department of Mathematics and Physics, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark
[2] Department of Biochemistry, Universität des Saarlandes, Saarbrücken, Federal Republic of Germany

Received March 18, 1991 / Accepted in revised form September 18, 1991

Abstract. The technique of Perturbed Angular Correlations of γ-rays has been used to study the rotational correlation times in aqueous solution of the peptides: oxytocin, glycyltryptophan, cholecystokinin and the glycopeptide ristocetin. These peptides were labelled with excited ^{111m}Cd through the covalent coupling of the metal chelator diethylenetriaminepentaacetic acid (DTPA) to the primary amines of the peptides. The experimental correlation times are in good accordance with calculations based on the molecular weight. This indicates that the ^{111m}Cd-DTPA is rigidly bound to the molecules. In the case of ristocetin, the correlation time was measured at 2 °C, 25 °C and 38 °C. These experiments show the expected linear dependence on the viscosity divided by temperature. The feasibility of determining rotational correlation times for peptides without lysines and with correlation times in the ns region is thus demonstrated. Also, the correlation time of ^{111m}Cd-DTPA coupled to the lysines of bovine serum albumin was determined. The measured correlation time is about 5 times less than the calculated correlation time. This effect is assigned to local motion. In spite of this, experiments show that ^{111m}Cd-DTPA-bovine-serum-albumin is significantly immobilised by aggregation with immunoglobulins. The nuclear quadrupole interactions, necessary for determining the correlations times, were determined for ^{111m}Cd-DTPA-ristocetin and ^{111m}Cd-DTPA-bovine-serum-albumin by adding sucrose to a concentration of 63% and cooling to 2 °C. This showed a small but significant difference between the two molecules. We interpret this as due to different conformations, possibly different coordination numbers.

Key words: Rotational diffusion – Oxytocin – Cholecystokinin – Ristocetin – Bovine serum albumin

Offprint requests to: E. Danielsen

Introduction

Proteins and peptides in aqueous solution undergo rotational diffusion as well as translational diffusion. As the rotational correlation time of a molecule is proportional to its volume, the detection of the correlation time can be used to study the interaction of molecules, e.g. aggregation or polymerisation.

A number of techniques have been developed for the detection of the rotational correlation time. Some of these are: dielectric relaxation, dynamic light scattering, NMR and fluorescence depolarization.

The technique of Perturbed Angular Correlations (PAC) is based on the detection of the time dependence of the angular correlation between two γ-rays emitted from the same nucleus. A review of the technique is given by Rinneberg (1979). The technique has two major advantages compared to other techniques: *1*) It is highly sensitive. A normal experiment uses about 0.2 pmol to 7 pmol of radioactive nuclei, depending on the half-life of the parent isotope. Hence, the actual sensitivity is limited by the amount of carrier metal introduced in the production of the PAC-isotope; *2*) The transparency of most biological tissue to γ-rays makes it possible to study molecular interactions betwen labelled molecules and molecules in inhomogeneous mixtures (blood samples etc.) or even in living creatures.

The use of PAC for detection of rotational correlation of proteins has so far been carried out mainly with nuclei in a specific metal binding site (Mullins and Kaplan 1983) or with the PAC-nuclei unspecifically bound to the protein (Leipert et al. 1968).

Here we have used the PAC-isotope ^{111m}Cd, specifically coupled to proteins and peptides through the covalent binding of the metal chelator, diethylenetriaminepentaacetic acid (DTPA), to the primary amines of the protein/peptide. In the present paper the nuclear quadrupole interaction of the ^{111*}Cd-DTPA-peptide complex is determined. Finally, we have investigated to what extent the observed correlation time reveals the overall molecular motion or local motion.

Materials and methods

Materials

Cholecystokinin (Formula weight 1064), glycyltryptophan (Formula weight 261), bovine serum albumin (BSA) (Molecular weight 66700), diethylenetriaminepentaacetic acid (DTPA) and oxytocin (Formula weight 1008) were from SIGMA, and ristocetin (Formula weight 2066) was a gift from Lundbeck A/S. Immunoglobulin-G (IgG) against BSA (antibody titre: 1 ml/900 μg BSA) was from Dakopatts. ^{108}Pd came from Oakridge. All other chemicals were of analytical purity.

Peptide preparation

Peptides and proteins were labelled with $^{111m}Cd^{2+}$ by coupling the bicyclic anhydride of DTPA to the terminal amino group and, in the case of BSA, mainly to the amino group of the lysines (Bauer et al. 1991 a). The labelling resulted in a stoichiometry of 2 DTPA molecules to one molecule BSA or ristocetin, respectively. All other peptides were labelled with a stoichiometry of 1:1. Unless otherwise stated, 10 nmol of labelled dehydrated peptide was dissolved in 0.1 M sodium acetate which had been purified for metal contamination by passing through a DTPA-gel (Bauer et al. 1991 a). For ristocetin at 38 °C experiments were carried out on concentrations from 76 nmol ml^{-1} to 607 nmol ml. Since the spectra gave the same time-integrated attenuation (for the definition of the time-integrated attenuation see Frauenfelder and Steffen 1965) these spectra were added and then analysed.

Preparation of radioactive ^{111m}Cd

The excited ^{111m}Cd was produced by irradiation of ^{108}Pd with 24-MeV α-particles at the cyclotron, Niels Bohr Institute. To keep contamination by other metal ions as low as possible, the palladium was electrolytically plated onto a graphite bar before activation. The electrolysis-bath corresponded closely to the recipe for heavy deposition of palladium by electrolysis (Parker 1983). The electrolysis produced a circular spot of about 11 mg palladium and 10 mm diameter.

The palladium plated graphite was irradiated by a 35 μA beam of 24 MeV, α-particles for 90 min, producing thereby about 370 MBq. The preparation of the electrolysis-bath and the separation of ^{111m}Cd from palladium after activation was carried out as described elsewhere (Bauer et al. 1991 b).

50–100 μl aqueous solution of $^{111m}Cd^{2+}$ was mixed with the solution of DTPA-labelled peptide and left to incubate for about 5 min. Any free ^{111m}Cd was then removed by passing the peptide through a DTPA-gel equilibrated in 0.1 M sodium acetate, pH 7. The distribution of the ^{111m}Cd-DTPA-peptide on various fractions caused a dilution of up to 50%. Less than 5% ^{111m}Cd was retained on the column, and the DTPA-column was, therefore, omitted in experiments with sucrose, in order to avoid an increase in the sample volume. For these experiments sucrose was mixed with ^{111m}Cd-DTPA-peptide and sufficient buffer to give a final concentration of 0.1 M sodium acetate, pH 7. The experiments on the DTPA-gel were carried out by adding the ^{111m}Cd solution to 1 g DTPA-gel equilibrated in 0.1 M sodium acetate, pH 7.

For one experiment an immunoprecipitate of IgG (BSA) and BSA was produced as follows: After passing the DTPA-gel, three fractions containing 9–10 nmol ^{111m}Cd-DTPA labelled BSA were pooled (3 ml total volume). This was then mixed with 1 ml IgG (BSA). After approximately 5 min the sample was centrifuged. 3.5 ml of the supernatant were removed, and the remaining 0.5 ml containing more than 90% of the activity was used for the PAC-experiment. The measurement was carried out at room temperature.

In all experiments the sample volume was increased from about 100 μl to 1 ml during the measurement. This was done in order to maintain an optimal count-rate.

The temperature, except room temperature, was controlled (±2 °C) by a Peltier element in thermal contact with the test tube. In one experiment the sample was submerged in liquid nitrogen.

PAC instrument

All experiments were carried out on a conventional fast-slow set-up using four BaF_2 detectors, (each 50.8 mm in diameter and length) arranged in a plane at fixed angles: 0° (1), 90° (2), 180° (3) and 270° (4), respectively. The typical electronic arrangement for PAC-experiments can be found in the review by Rinneberg (1979).

Eight different spectra were collected; four forward in time and four in reverse. The perturbation function, $G_2(t)$ was derived as follows:

$$A_{2\text{eff}}\,G_2(t) = \frac{\sqrt{W_{42}\cdot W_{31}} - \sqrt{W_{41}\cdot W_{32}}}{\sqrt{W_{42}\cdot W_{31}} + 2\sqrt{W_{41}\cdot W_{32}}} + \frac{\sqrt{W_{24}\cdot W_{13}} - \sqrt{W_{23}\cdot W_{14}}}{\sqrt{W_{24}\cdot W_{13}} + 2\sqrt{W_{23}\cdot W_{14}}} \quad (1)$$

where W_{ij} denotes the coincidence spectrum after background subtraction (and time reversal of the first four spectra) of 150 keV γ-rays in detector i and 247 keV γ-rays in detector j.

The unperturbed amplitude of 16% (Haas and Shirley 1973) was reduced to an $A_{2\text{eff}}$ of about 10% due to a combination of the finite sizes of detectors and source, misalignment, and absorption.

Before each experiment the spectrometer was time calibrated and the time resolution (about 1.9 ns) was determined using a ^{75}Se source.

Data-analysis

The spectra were analyzed by a least χ^2 fit. The perturbation function is characterized by the parameters ω_0, η, δ

and τ. (An intermediate state of spin 5/2 is assumed in the following.)

ω_0 is the strength of the electric field gradient defined as:

$$\omega_0 = 12\pi |V_{zz} e Q|/(40 h) \quad (2)$$

Q is the nuclear quadrupole moment, η is the asymmetry parameter defined as $\eta = (V_{xx} - V_{yy})/V_{zz}$, where V_{aa} denotes the a'th diagonal element of the electric field gradient tensor in the coordinate system chosen such that the tensor is diagonal and $|V_{zz}| \geq |V_{yy}| \geq |V_{xx}|$. If the electric field gradient varies for different nuclei due to small statistical variations in the surroundings, this can often be described as a Gaussian frequency distribution. The relative width $(\Delta\omega_0/\omega_0)$ of the Gaussian distribution is denoted δ.

The corresponding perturbation function for randomly oriented molecules with time independent electric field gradients (i.e. frozen solution) is:

$$G_2^{\infty}(t) = a_0 + \sum_{i=1}^{3} a_i \exp(-0.5\,(\omega_i t\,\delta)^2)\cos(\omega_i t) \quad (3)$$

where a_0 and a_i depend on η, and ω_i depend on η and ω_0 (Bauer 1985). a_0 is termed the hardcore value.

Two models exist for describing the effect on the PAC-spectra of reorientation of the molecules: the Debye model and the strong collision model. In the Debye model the reorientation is a small step process characterized by the diffusion parameter θ. The corresponding correlation time is $\tau_c = 1/6\,\theta$ (assuming isotropic rotational diffusion). For a spherical molecule of volume V the diffusion parameter, θ is as follows (Debye 1929):

$$\theta = kT/(6\xi V) \quad (4)$$

where ξ is the viscosity of the solution and thus, the calculated correlation time (measured by $G_2(t)$ is:

$$\tau_c = \xi V/(kT) \quad (5)$$

The diffusion tensor of an ellipsoid of revolution has also been derived (Perrin 1934). In the strong collision model the molecules undergo random reorientations. The probability that a molecule undergoes a reorientation in the time interval dt is dt/τ_c.

For slowly reorienting molecules, the result of the adiabatic approximation of the Debye model is (Marshall et al. 1972):

$$G_2^{\tau}(t) = \exp(-t/\tau_c)\,G_2^{\infty}(t) \qquad (\omega_0\,\tau_c >> 1) \quad (6)$$

where $G_2^{\infty}(t)$ is the perturbation function of the static interaction. For strong collisions (6) is only an approximation, though it seems unlikely that it will be possible to distinguish the two models experimentally (Danielsen and Bauer 1991)

The expression for fast reorienting molecules (Abragam and Pound 1953), generalized to asymmetric electric field gradients (Huntress 1968), was applied to molecules with short correlation times. In the case where the intermediate state has $I = 5/2$, it takes the following form:

$$G_2^{\tau}(t) = \exp[-2.8\,\omega_0^2\,t\,\tau_c\,(1+\eta^2/3)] \qquad (\omega_0\,\tau_c \ll 1,\ \delta = 0) \quad (7)$$

(This expression is valid for the Debye model as well as the strong collision model.)

A generalised expression of (7) for non-zero frequency-distribution (Danielsen and Bauer 1991) was used in the analysis of spectra with short correlation times.

Most of the spectra in the present paper have, however, correlation times in the intermediate region where the Debye model gives no analytic expression. We therefore used the strong collision model, first applied to PAC by Scherer and Blume (Scherer 1970; Blume 1971). The numerical technique for computing $G_2^{\tau}(t)$ (Dattagupta 1981), was used to form a database of $G_2(\tau, \omega t, \eta)$ from which the $G_2^{\tau}(t)$ was calculated. Details of this computational method are given elsewhere (Danielsen and Bauer 1991). The use of this model instead of the Debye model may lead to an error of up to 20% in the determination of the correlation time for long correlation times.

Results

The nuclear quadrupole interaction was determined for ^{111m}Cd-DTPA coupled to BSA, ristocetin and the DTPA-gel, respectively (see Fig. 1). To slow down the rotational diffusion in the case of BSA and ristocetin, the experiments were carried out in 63% sucrose (w/w) and, in the case of BSA, also in 50% sucrose. The spectrum of the ^{111m}Cd-DTPA-gel was collected at $-18\,°C$ and at liquid nitrogen temperature. The results of a least χ^2 analysis are listed in Table 1.

The immobilization of the DTPA-gel at liquid nitrogen temperature is seen as the decay of the perturbation function to the hardcore value, a_0 in Fig. 1.C, whereas the motion of the molecules in sucrose makes the perturbation function decay to 0 in Fig. 1.A and 1.B (see (3) and (6))

The rotational correlation was measured for ristocetin in aqueous solution (0.1 M sodium acetate, pH 7) at 2 °C, 25 °C, and at 38 °C, and for glycyltryptophan, oxytocin, cholecystokinin and BSA at 2 °C. Some of these spectra are shown in Figs. 2 and 3. The ristocetin spectra were analysed using the nuclear quadrupole interaction determined from ristocetin at 2 °C in 63% sucrose. The BSA spectra were analysed likewise with the parameters determined for BSA at 2 °C in 63% sucrose. All other spectra were analysed with the nuclear quadrupole interaction of BSA as well as that of ristocetin. This was done in order to determine the uncertainty in the rotational correlation time due to the ambiguity of the nuclear quadrupole interaction. The results are shown in Table 2 and Fig. 4.

The effect of immunoprecipitation of BSA with IgG at 25 °C is shown in Table 2 and Fig. 3.

Discussion

The nuclear quadrupole interaction

The spectra listed in Table 1 can be used to determine a nuclear quadrupole interaction in terms of a strength (ω_0), an asymmetry parameter (η) and a frequency distribution (δ). As illustrated by the Fourier transforms in

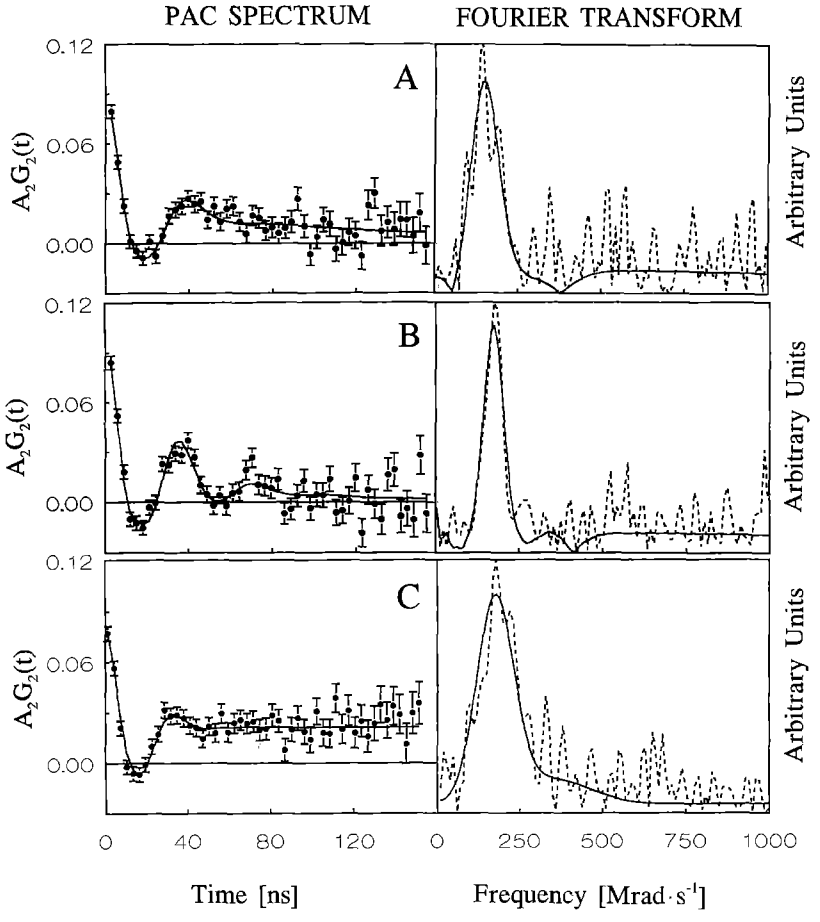

Fig. 1A–C. Perturbation functions (*left*) and Fourier transforms (*right, broken line*) of spectra used for determining the nuclear quadrupole interaction. The full line is the result of a least-χ^2 fit. The Fourier transform of the best fit to the perturbation function is also shown as a full line. The experimental points represent the average of 4 experimental points and the bars indicate standard deviations. The Fourier transforms were carried out after subtracting $a_0 \exp(-t/\tau_c)$ from the pertubation function. **A** BSA, 2 °C, 63% sucrose. **B** ristocetin, 2 °C, 63% sucrose. **C** DTPA-gel submerged in liquid nitrogen

Table 1. Nuclear quadrupole interactions for various ^{111*}Cd-DTPA-complexes

	η	ω_0 [Mrad · s^{-1}]	δ	τ_c [ns][a]
BSA, 63% sucrose, 2 °C	0.75 ± 0.14	91.1 ± 5.2	0.25 ± 0.05	130.0 ± 30.0
BSA, 50% sucrose, 2 °C	0.75 (fixed)	91.1 (fixed)	0.25 (fixed)	56.0 ± 9.0
Ristocetin, 63% sucrose, 2 °C	1.00 + 0/−0.12	98.7 ± 2.5	0.14 ± 0.02	47.0 ± 14.0
DTPA-gel, −18 °C	1.00 (fixed)	98.7 (fixed)	0.14 (fixed)	26.0 ± 4.0
DTPA-gel, Liquid Nitrogen	1.00 (fixed)	98.7 (fixed)	0.32 ± 0.03	∞ (fixed)

[a] The first four spectra listed were analyzed in the strong collision model (Danielsen and Bauer 1991)

Table 2. Rotational correlation of peptides and lysine labelled BSA determined by PAC

	m_w (labelled molecule)	$\tau_{experimental}$ [ns][a]	Model used in data-analysis	$\tau_{calculated}$ [ns][b]
Ristocetin, 2 °C	3028	2.0 ± 0.1	strong collision	2.33
Ristocetin, 25 °C	3028	0.67 ± 0.03	Equation 7	1.16
Ristocetin, 38 °C	3028	0.51 ± 0.05	Equation 7	0.86
Glycyltryptophan, 2 °C	742	0.57 ± 0.03	Equation 7	0.57
Oxytocin, 2 °C	1489	0.62 ± 0.03	Equation 7	1.14
Cholecystokinin, 2 °C	1545	0.75 ± 0.04	Equation 7	1.19
BSA, 2 °C	67700	10.4 ± 1.3	strong collision	52.1
BSA + IgG against BSA, 25 °C		41.7 ± 5.2	strong collision	

[a] The experiments except BSA were analyzed using the nuclear quadrupole interaction of ristocetin (Table 1). Analyzing the spectra of glycyltryptophan, cholecystokinin and oxytocin using the nuclear quadrupole interaction of BSA, increased τ_c by a factor of 1.45

[b] Theoretical values of the correlation time, based on (5) using the viscosity of water and a hydrated volume of $1.07 \cdot 10^{-3}$ m^3 kg^{-1} times the mass of the molecule in kg

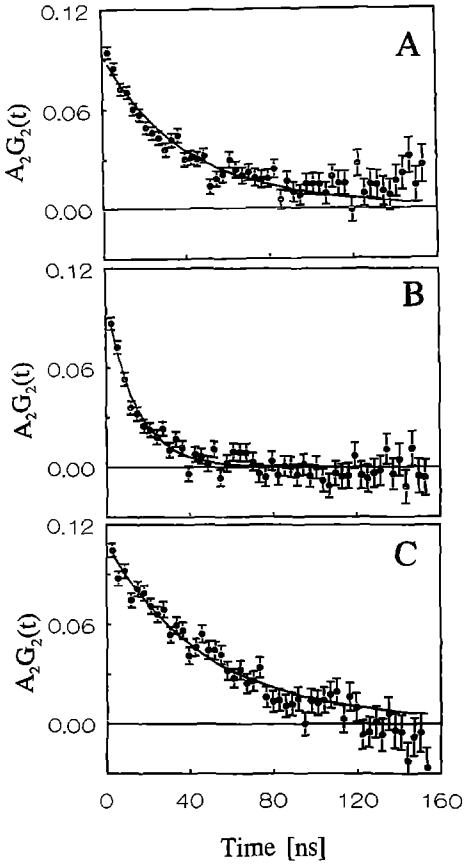

Fig. 2A–C. Perturbation functions showing the effect of changing the temperature and molar weight, respectively. Display as in Fig 1, left. **A** ristocetin aqueous solution, 25 °C. **B** ristocetin aqueous solution, 2 °C. **C** glycyltryptophan, 2 °C

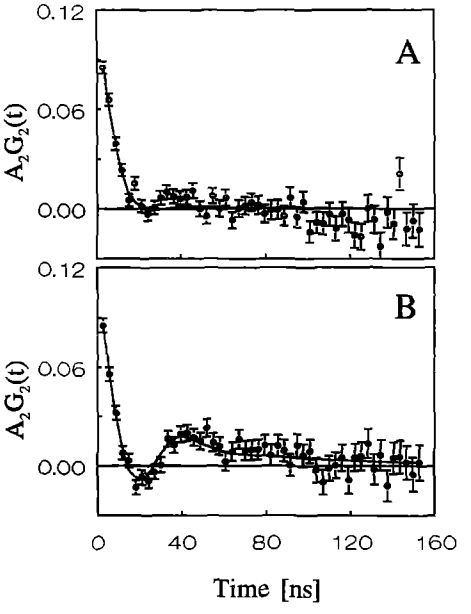

Fig. 3A, B. Perturbation functions showing the effect of adding IgG to ^{111m}Cd-DTPA-labelled BSA. Displayed as in Fig 1, left. **A** BSA in 0.1 M sodium acetate (pH 7) at 2 °C. **B** precipitate of about 10 nmole BSA in 3 ml 0.1 M sodium acetate (pH 7) to which was added 1 ml IgG(BSA), at room temperature (25 °C)

Fig. 1, two different conformations are necessary for explaining the spectra: one describing the DTPA-Cd complex when DTPA is coupled to BSA, the other describing the complex when DTPA is coupled to either ristocetin or the gel (Table 1). The different conformations of the DTPA-Cd-complexes could be due to differences in coordination numbers in the various situations. One reason for this might be that the presence of hydroxyl groups in the vicinity of the DTPA-group causes one of the four carboxyls of DTPA to hydrogen bond of the hydroxyl group. Characteristically, ristocetin has many hydroxyl groups, but also the link between DTPA and the gel contains a hydroxyl group that seems free to bind. The side group of the lysines, however cannot form a hydrogen bond.

The strength and the asymmetry of the nuclear quadrupole interaction can be calculated using the Angular Overlap Model (Bauer et al. 1988). In this model each ligand contributes an axially symmetric field gradient with the symmetry axis through the ligand and the metal ion. Each ligand is assigned a partial nuclear quadrupole interaction parameter, and the resulting nuclear quadrupole interaction is then determined by addition of the contributions from all the ligands and diagonalisation of the resulting matrix. The partial nuclear quadrupole interaction parameter has been determined for a carboxyl group coordinating through one oxygen, but not for a tertiary amine.

For octahedral symmetry the calculation is particularly simple: The contribution of a ligand positioned along the positive or negative part of the x-axis is (Bauer et al. 1988):

$$\overline{\overline{\omega}}_{\text{lig}} = \begin{pmatrix} \omega_{\text{lig}} & 0 & 0 \\ 0 & -0.5\,\omega_{\text{lig}} & 0 \\ 0 & 0 & -0.5\omega_{\text{lig}} \end{pmatrix} \tag{8}$$

where ω_{lig} is the partial nuclear quadrupole interaction parameter of that particular ligand.

If one carboxyl group of DTPA hydrogen bonds to a protein hydroxyl group, then six metal ligands will be free to coordinate to Cd^{2+}, and assuming octahedral structure, two conformations must be considered:

A) The three nitrogens form a plane containing the Cd^{2+}-ion. This configuration will yield $\eta = 1$ and $\omega_0 = 1.5|\omega_n - \omega_{ox}|$, where ω_n and ω_{ox} denote the partial nuclear quadrupole interaction parameters of the nitrogen and oxygen ligands, respectively.

B) The three nitrogens form a pyramid (all three Cd-N bonds at right angles). This configuration will yield $\omega_0 = 0$ and η undefined.

In the example *A*) above, three nitrogens are located in the x, -x and y directions, and three oxygens are located in the -y, z and -z directions. This gives a sum matrix with the diagonal elements:

$$\omega_{xx} = 2\omega_n - 0.5(\omega_n + 3\omega_{ox}) = 1.5(\omega_n - \omega_{ox})$$

$$\omega_{yy} = \omega_n + \omega_{ox} - 0.5(2\omega_n + 2\omega_{ox}) = 0$$

$$\omega_{zz} = 2\omega_{ox} - 0.5(\omega_{ox} + 3\omega_n) = -\omega_{xx}$$

resulting in, $\eta = 1$ and $\omega_0 = 1.5|\omega_n - \omega_{ox}|$. Case *B*) can be solved in a similar manner.

The value of η measured for ristocetin in 63% sucrose at 2 °C is in accordance with conformation *A*). If one uses the known partial nuclear quadrupole interaction parameter of the carboxyl group, $\omega_{ox} = 245$ Mrad · s^{-1} (Bauer et al. 1988), this requires that the value of the partial nuclear quadrupole interaction parameter of a tertiary amine is either 311 or 179 Mrad · s^{-1}. Probably a partial nuclear quadrupole interaction parameter of 311 Mrad · s^{-1} can be excluded since: The partial nuclear quadrupole interaction parameter known so far for ligands coordinating to Cd^{2+} through nitrogen has the following values in Mrad · s^{-1}: 139 for a primary amine, 190 for a hydrazine and 95 for an imidazole. The only ligand so far known to give a higher partial nuclear quadrupole interaction parameter than 250 Mrad · s^{-1} is thiocyl with 330 Mrad · s^{-1} (Bauer et al. 1988).

Using the partial nuclear quadrupole interaction parameter of 179 Mrad · s^{-1} for the tertiary amine, the Angular Overlap Model can be used to calculate the nuclear quadrupole interaction for the Cd-complex assuming that all four carboxyl groups coordinate as well as the three amines. Calculations show that it possible to find conformations with a calculated nuclear quadrupole interaction as that observed for BSA in 63% sucrose at 2 °C.

The nuclear quadrupole interaction of the DTPA-gel at −18 °C shows a significant motion (Table 1). This motion is immobilised by submerging the sample in liquid nitrogen. The latter condition also gives rise to a large increase in frequency distribution. This frequency distribution could either be due to contributions to the electric field gradient by the dipole moment of the neighbouring water molecules (a contribution that could be averaged out by the motion at higher temperatures), or it could indicate that freezing of the local water distorts the configuration of the ^{111m}Cd-DTPA complex.

In all of the cases listed in Table 1, the frequency distribution, δ, is high as compared to studies of crystallized cadmium complexes (Bauer et al. 1988) and ^{111m}Cd-substituted in the zinc sites of enzymes (Bauer 1985). For the cadmium complexes in the crystalline state and for carboxypeptidase and superoxide dismutase no frequency distribution was observed and for alcohol dehydrogenase and carbonic anhydrase a frequency distribution of 10 to 15% was found. A high value of δ probably reflects a higher freedom of the positions of the ligands. This freedom is limited by the crystal structure of the cadmium complexes, and by the protein structure in some enzymes, but not completely by the structure of the DTPA-Cd complex. The high η together with the faster motion of the molecules (compared to the enzymes) makes the determination of η and ω_0 less accurate as reflected in the high uncertainties in Table 1. However these uncertainties have little effect on the determination of the correlation times of the molecules in water, and no effect on the relative correlation times.

Correlation times of cholecystokinin, glycyltryptophan, oxytocin and ristocetin

The models used for interpreting the perturbation functions assume that reorientation is isotropic. This cannot be distinguished from anisotropic reorientation in the fast rotational limit, but any deviation from spherical symmetry tends to make the effective correlation time longer, which can be seen by inserting the friction coefficients (Perrin 1934) into the equations for relaxation times measured by NMR (Huntress 1968). It has been shown that these equations can be applied to calculations of perturbed angular correlations (Marshall et al. 1972). For slow rotational diffusion, anisotropy of the rotational diffusion complicates the correlation function (Marshall et al. 1972). However, the tendency will be the same, namely, as follows: if one analyses the spectrum using a single correlation time, deviation from spherical symmetry will give a longer average correlation time as compared to the correlation time of a spherical molecule of the same mass. In contrast to this, local flexibility will decrease the correlation time.

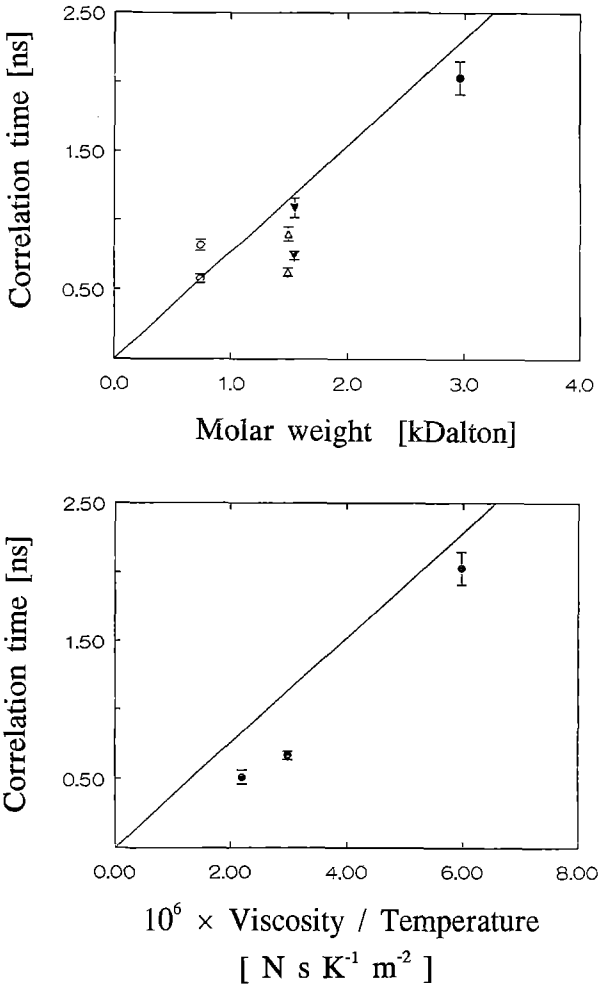

Fig 4. The experimental correlation times are shown as a function of molar weight (*above*) and viscosity divided by temperature (*below*). The molar weight used is that of the labelled molecule. (For each ^{111m}Cd-DTPA group the molar weight is increased by 481.) The full lines indicate the calculated values using (5), the viscosity of water and a hydrated volume of 1.07 10^{-3} m^3 kg^{-1} times the mass of the molecule in kg. For glycyltryptophan (o), oxytocin (Δ) and cholecystokinin (▼), the correlation time determined using the quadrupole interaction of BSA (highest value) is shown as well as the result using the quadrupole interaction of ristocetin. Ristocetin is indicated by ●

Figure 4 compares the calculated correlation time (5) to the measured correlation time of the peptides at 2 °C. For the calculation the viscosity of water was used. ($1.644 \cdot 10^{-3} \cdot N \cdot s \cdot m^{-2}$ at 2 °C, $0.891 \cdot 10^{-3} \cdot N \cdot s \cdot m^{-2}$ at 25 °C and $0.685 \cdot 10^{-3} \cdot N \cdot s \cdot m^{-2}$ at 38 °C.) The volumes of the molecules were calculated using a specific volume of $0.725 \cdot 10^{-3} \cdot m^3 \cdot kg^{-1}$ and a hydration of 0.345 g H_2O/g protein. These values are averaged over listed specific volumes and hydrations (Kuntz and Kauzmann 1974). With the exception of ristocetin, the spectra have been analysed using the nuclear quadrupole interaction of BSA as well as the nuclear quadrupole interaction of ristocetin. Both results are given. (The longest correlation time is the result of analysing the spectrum with the nuclear quadrupole interaction of BSA.)

Figure 4 also shows the temperature dependence of the correlation time of ristocetin. Some deviation from the calculated values is expected due to variations of hydration and local flexibility. Generally, the observed values are in good accordance with the calculated values. Therefore the DTPA molecules appear to be rigidly coordinated to the molecules.

The rotational correlation time of the tyrosine of oxytocin has been measured by frequency-domain fluorometry (Lakowicz et al. 1987). The measured correlation times are 29 ps and 454 ps measured at 25 °C. The longer correlation time is interpreted as the global reorientation. The correlation time of oxytocin has also been studied by the method of oxygen quenching and steady state anisotropy measurements (Maliwal and Lakowicz 1986). This method yielded only the global correlation time: 180 ps, also at 25 °C. In order to compare the correlation time measured by PAC at 2 °C to the correlation time measured by fluorescence anisotropy decay, the effect of temperature and viscosity must be taken into account as well as the effect of the larger mass of the ^{111m}Cd-DTPA labelled molecule. The correlation time of 624 ps measured by PAC at 2 °C corresponds to 211 ps for the unlabelled molecule at 25 °C. Thus, there is good agreement between the global correlation times determined by ^{111m}Cd-PAC and the 180 ps correlation time mentioned above.

Correlation time of bovine serum albumin

BSA is probably one of the most frequently studied molecules in terms of hydrodynamic properties. A comparison of different methods (Kuntz and Kauzmann 1974) shows that it is somewhat asymmetric with an axial ratio of 3 and a hydration of about 0.4 g H_2O/g protein. It has a molecular weight of 66700 (Squire et al. 1968). This gives a calculated τ_c of 53 ns for the equivalent spherical molecule, which must be compared to the observed τ_c of 10.4 ns. The experimental diffusion is thus about 5 times faster than the calculated. A correlation time shorter than the calculated is also observed in the sucrose experiments (see the discussion below). This fast motion observed in ^{111m}Cd-DTPA-BSA could originate from motional flexibility of the lysine side chain to which the DTPA group is coupled. Local motion of a number of different fluorophores all coupled to lysines of BSA has been measured by fluorescence anisotropy decay (Burghardt 1983). The motion of the fluorophore with respect to the protein could be described by rotational diffusion constants corresponding to correlation times ranging from 26 ns (dansyl) to .13 ns (eosin), measured at room temperature. (The correlation time is derived from $d_{3,3}$ as $1/(6\,d_{3,3})$, where $d_{3,3}$ is the value listed by Burghardt for the rotational diffusion constant of the fluorophore coupled to BSA in solution.) Thus the local motion depends on the labelling group.

For PAC the combined effect of local and global motion depends on whether the local motion is isotropic or whether it is anisotropic in such a way that the time averaged electric field gradient is non-zero (for example wobling in a cone). The first case can be treated if also the global motion is isotropic, then $1/\tau_{\text{measured}} = 1/\tau_{\text{BSA}} + 1/\tau_{\text{Lysine}}$. The anisotropic local motion has not been treated yet, except in the case where it is very fast. (The analogy of the condition in equation 7 is: $\tau_c' \, 12\pi \, |V_{zz}' \, e \, Q|/40\,\text{h} \ll 1$, where V_{zz}' is the largest element of the diagonalised tensor formed as the difference between the electric field gradient tensor and the time averaged electric field gradient tensor and τ_c' is the correlation time of the local motion.) In that limit the measured τ_c will be reduced by a factor of $(\omega_0/\langle\omega_0\rangle)^2$ relative to the calculated. It is not possible to distinguish between these two extremes by a single experiment, but the matter could be investigated by repeating the experiment with a protein of a different mass than BSA. In the first case, there will be almost no influence of the volume on the detected τ_c. In the second case τ_c will be proportional to the volume, since the protein will tumble as if the electric field gradient was reduced but fixed with respect to the orientation of BSA.

Aggregation of BSA with IgG

The interaction with IgG decreased the correlation time by a factor of 4 in spite of the fact that the temperature was raised to 25 °C. The interaction with IgG thus clearly slows down the motion. The effect on motion by the formation of an aggregate or a gel structure may be complex, as it slows down the overall motion but probably also local motion. It is not evident whether a similar decrease in motion would occur in an in vivo situation, where the immunoglobulins do not cause aggregation. However the reaction can definitely be detected by PAC, thus offering many possible applications in the field of immunology as well as for studies of hormone-receptor interactions.

Correlation times in sucrose

The macroscopic viscosity of sucrose is 0.39 $N \cdot s \cdot m^{-2}$ for 63% sucrose at 2 °C and 0.040 $N \cdot s \cdot m^{-2}$ for 50% sucrose at 2 °C (sixth order polynomial interpolation from tabulated values) (Fasman 1988).

The correlation times of the global reorientation of the molecules calculated by (5) are: Ristocetin in 63% sucrose: 560 ns, BSA in 63% sucrose: 12000 ns and BSA in 50% sucrose 1300 ns. A comparison with the results listed in Table 1 clearly shows that a motion remains which is not as sensitive to the viscosity as predicted by (5).

Bucci and Steiner have discussed the effect of sucrose on correlation times measured by fluorescence anisotropy decay (Bucci and Steiner 1988). They suggest that the localized motion of a fluorophore may be less sensitive to the viscosity than the global reorientation for two reasons: Either the local viscosity could be different than the bulk viscosity or the reorientation of the fluorophore could involve transition between different energy minima with a rate governed by the probability of release from a position of the minimum energy. This rate could be more or less viscosity independent. In the case of fluorescence anisotropy decays, the interpretation is facilitated by the possibility of detecting different correlation times reflecting different reorientation processes. This is generally not possible for PAC-spectra. The correlation times in Table 1 thus could represent the overlay of at least two different motions: a global reorientation and one ore more localized motions. Based on the present data we are not able to determine the nature of the localized motion more accurately. However, it is clear that this local motion is influenced by the viscosity indicating that the label is on the surface of the protein. It is also evident that local motion can indeed be detected by PAC. Future improvements of the theory together with more detailed experiments could offer the technique as a tool for studying local motion as well as global reorientation.

Though the local motion apparently does not overshadow the global reorientation of the peptides in aqueous solution, it may account for the tendency of the measured correlation time to be shorter than the correlation time as illustrated by Figs. 1 and 2 and Table 2.

Choice of isotope

The isotope ^{111m}Cd was chosen for two reasons. First, the intermediate lifetime of 121 ns together with the nuclear quadrupole interaction of the order of 100 MRad/s makes it suitable for investigating correlation times in the range of 0.1 ns to 200 ns and possibly even longer times. This makes the isotope very useful for detecting correlation times of proteins and peptides in aqueous solution. Second, the decay of ^{111m}Cd to the intermediate state does not give rise to the after effects seen in spectra measured using ^{111}In (Bauer et al. 1991, a). The main disadvantage of ^{111m}Cd is the rather short half life of the excited level (49 m), which means that sample preparation has to be carried out within a few hours. It is possible that this problem can be overcome by using the isotope ^{111}In for experiments that demand very long preparation times (i.e. in vivo experiments) or very low amounts of materials. Comparative studies using ^{111}In and ^{111m}Cd respectively could then be used to determine the correlation times of the experiments where ^{111m}Cd can not be used directly.

Conclusion

The present work shows that rotational diffusion of small peptides can be studied by ^{111m}Cd-PAC. For larger proteins such as BSA or smaller petides in sucrose solution (ristocetin) it is possible to detect local motion by ^{111m}Cd PAC. The PAC spectrum, after immunoreaction of BSA with its polyclonal antibody, is clearly different from the PAC spectrum of BSA without the antibody. A similar observation has been made with cholecystoknin using ^{111}In (Bauer et al. 1991 a). This gives hope for using the PAC method as a diagnostic tool for hormone-receptor interaction in vivo and in vitro although there still is a need for a quantitative understanding of the motional part connected to the local flexibility.

Acknowledgements. We wish to thank the cyclotron staff at the Niels Bohr Institute for the production of ^{111m}Cd. Parts of the PAC spectrometer were granted by Brd. Hartmann's Foundation. The present work was carried out under the Danish Biotechnology programme and was supported by the Danish Natural Science Research Council.

References

Abragam A, Pound RV (1953) Influence of electric fields on angular correlations. Phys Rev 92:943–962

Bauer R (1985) Perturbed angular correlation spectroscopy and its application to metal sites in proteins: possibilities and limitations. Q Rev Biophys 18:1–64

Bauer R, Jensen SJ, Schmidt-Nielsen B (1988) The angular overlap model applied to the calculation of nuclear quadrupole interactions. Hyperfine Interact 39:203–234

Bauer R, Atke A, Danielsen E, Marcussen J, Olsen CE, Rehfeld J, Saermark T, Schneider D, Vilhardt H and Zeppezauer M (1991 a) The potential of perturbed angular correlation of gamma rays as a tool for dynamic studies of peptides/proteins. App Rad Isotopes (in press)

Bauer R, Bjerrum MJ, Danielsen E, Kofod P (1991, b) Coordination geometry of cadmium at the zinc and copper sites of superoxide dismutases: a study using perturbed angular correlations of γ-rays from exited ^{111}Cd. Acta Chem Scand (Ser A) 45:593–604

Blume M (1971) Perturbed angular correlations: perturbation factor for arbitrary correlation time. Nucl Phys A167:81–86

Bucci E, Steiner RF (1988) Anisotropy decay of fluorescence as an experimental approach to protein dynamics. Biophys Chem 30:199–224

Burghardt TP (1983) Fluorescence depolarization by anisotropic rotational diffusion of a luminophore and its carrier molecule. J Chem Phys 78:5913–5919

Danielsen E, Bauer R (1991) Analysis of perturbed angular correlation spectra of metal ions bound to proteins with rotational correlation times in the intermediate region. Hyperfine Interact 62:311–324

Dattagupta S (1981) Study of time-dependent hyperfine interactions by PAC, Mössbauer effect, μSR and NMR: review of stocastic models. Hyperfine Interact 11:77–126

Debye P (1929) Polar molecules Dover, New York

Fasman GP (1988) Handbook of biochemistry and molecular biology, 3rd edn. Physical and Chemical data, vol I CRC Press, Boca Raton, Florida

Frauenfelder H, Steffen RM (1965) Angular distribution of nuclear raditon. In: Siegbahn K (ed) Alpha-beta- and gamma-ray spectroscopy, vol 2. North-Holland, Amsterdam, pp 997–1198

Haas H, Shirley DA (1973) Nuclear quadrupole interaction studies by pertubed angular correlations. J Chem Phys 58:3339–3359

Huntress WT (1968) Effects of anisotropic molecular rotational diffusion on nuclear magnetic relaxation in liquids. J Chem Phys 48: 3524–3533

Kunz ID, Kauzmann W (1974) Hydration of proteins and polypeptides. Adv Protein Chem 28: 239–345

Lakowicz JR, Laczko G, Gryczynski I (1987) Picosecond resolution of Tyrosine fluorescence and anisotropy decays by 2 GHz frequency-domain fluorometry. Biochemistry 26: 82–90

Leipert TK, Baldeschwieler JD, Shirley DA (1986) Applications of gamma ray angular correlations to the study of biological macromolecules in solution. Nature 220: 907–909

Maliwal BP, Lakowicz JR (1986) Resolution of complex anistropy decays by variable frequency phase-modulation fluorometry: a simulation study. Biochem Biophys Acta 873: 161–172

Marshall AG, Werbelow LG, Meares CF (1972) Effect of molecular shape and flexibility on Gamma-ray directional correlations. J Chem Phys 57: 364–370

Mullins OC, Kaplan M (1983) Perturbed angular correlation studies of indium metalloporphyrin complexes. J Chem Phys 79: 4475–4488

Parker EA (1983) Palladium plating. Metal Finishing '83: 294–296

Perrin F (1934) Mouvement brownien d'un ellipsoide (I). Dispersion diéléctique pour des molecules ellipsoidales. J Phys 10: 497–511

Rinneberg HH (1979) Applications of perturbed angular correlations to chemistry and related areas of solid state physics. Atomic Energy Rev 172: 477–595

Scherer C (1970) Gamma-gamma angular correlations: a model for statistical perturbation with any correlation time. Nucl Phys A157: 81–92

Squire PG, Moser P, O'Konski CT (1968) The hydrodynamic properties of bovine serum albumin monomer and dimer. Biochemistry 12: 4261–4272