

Accelerated Hydrolysis of Aspirin Using Alternating Magnetic Fields

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Abstract The major problem of current drug-based therapy is selectivity. As in other areas of science, a combined approach might improve the situation decisively. The idea is to use the pro-drug principle together with an alternating magnetic field as physical stimulus, which can be applied in a spatially and temporarily controlled manner. As a proof of principle, the neutral hydrolysis of aspirin in physiological phosphate buffer of pH 7.5 at 40 °C was chosen. The sensor and actuator system is a commercially available gold nanoparticle (NP) suspension which is approved for animal usage, stable in high concentrations and reproducibly available. Applying the alternating magnetic field of a conventional NMR magnet system accelerated the hydrolysis of aspirin in solution.

Keywords Gold nanoparticle · Magnetic field · Relaxation · Hydrolysis · Pro-drug

Introduction

The biggest problem of drug-based therapy is selectivity because of side effects that can be dose-limiting. This challenge led to different strategies with chemically increased selectivity (pro-drug approach), and physically increased selectivity (physical stimuli such as lasers) [1].

The latter approach, hyperthermia, is used as sole agent or as an adjuvant therapy together with chemotherapy and radiotherapy [2], utilizing magnetic fields [3] and NIR lasers [4]. Despite promising results, optical energy sources

are mainly limited to the treatment of subcutaneous tumours. As in other areas of science, a combination of two effects might improve the situation decisively. The idea is to use the pro-drug principle together with an alternating magnetic field as physical stimulus, which can be applied spatially and temporarily controlled. Moreover, it is not limited to surfaces and delivers very small amounts of energy to the target, thereby avoiding thermal damage in non-treated areas.

Experimental Section

NMR Experiments

As standard pulse program, the water suppression Watergate W5 pulse sequence with gradients using double echo was taken [5]. It was modified for stimulating the hydrolysis of aspirin with a loop 1,000 times over a z-gradient of 100 μ s length, of rectangular shape, 10% of maximum power, and alternating in both directions with a delay of 50 μ s between each gradient. This sequence was looped 10 times with a delay of 100 ms in between, after which the standard pulse sequence started. A total of 512 scans were accumulated which resulted in a total experimental time of 45 min and 16 s (relaxation delay set to 0.7 s). After this modified pulse sequence, the standard pulse program with 16 scan was used to record the spectra for the analysis of the hydrolysis. Temperature measurements before and after applying the alternating gradient using an internal calibration system [6] resulted in a difference of 0.9 K for a sample without gold nanoparticle (NP), and 0.7 K for a sample with gold NP. Thus, the bulk temperature was kept constant. Measurements of pH were conducted before and after applying the alternating gradient. The pH remained

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constant (difference below 0.05). Furthermore, in the neutral pH range applied, the hydrolysis of aspirin is hardly affected from small pH variations [7]. The spinning rate was 20 Hz in all measurements.

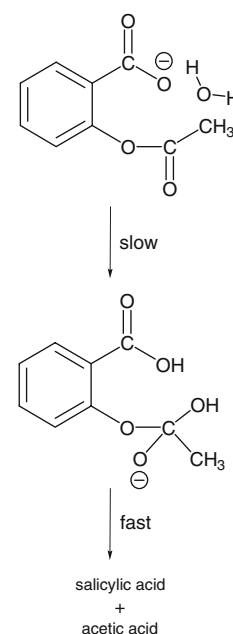
Statistical Analysis

Two types of samples (A: 1 mM aspirin alone, B: 1 mM aspirin plus a gold NP suspension with a total amount of gold of 40 mg which translates roughly to 10^{17} NP in a final volume of 0.6 mL) were treated with two hydrolysis conditions (1: without, 2: with an alternating magnetic field, resulting in four combinations: A1, A2, B1 and B2). Two sub-groups of the data were formed: the first group consists of the data from A1, A2, and B1 (six data points for each sampling time), and represent the hydrolysis reaction without the combined effect of gold NP and the alternating field. The second group consists of B2 data (four data points for each sampling time). The homogeneity of variances between the two groups at the three different sampling times was checked with the F-test (Excel™ worksheet). At all three sampling times there is homogeneity on the 0.01 level. This justifies the following analysis of variance (ANOVA). The null-hypothesis is that there is no significant deviation between group 1 and group 2. The critical values are 11.26 at the 0.01 level, and 25.41 at the 0.001 level. The calculated *F*-values are 17.58 at 47 min, 39.15 at 94 min, and 47.4 at 141 min. This shows that the hypothesis can be rejected on a 0.001 level of significance, and hence, there is a significantly increased hydrolysis for the combination of gold NP and an alternating magnetic field. To further support this statistical analysis, it was also tested if there is a significant difference between the direct pairs of A1 and B1, and A2 and B2, which would indicate the influence of gold NP alone. In this case, the ANOVA shows no significant differences. Additionally, the influence of the alternating magnetic field was tested with ANOVA. Again, all calculated *F*-values are below the critical values. The standard deviations were calculated for the two groups of data and are shown as error bars in Fig. 3 of the main text. To summarize, the statistical analysis showed that (i) the basis for the analysis of variance (homogeneity of variances) is given, (ii) only the combination of gold NP and an alternating magnetic field increases significantly at a 0.001 level the hydrolysis of aspirin, and (iii) neither gold NP nor alternating magnetic fields alone lead to a significant effect on the hydrolysis.

Results and Discussion

In physiological phosphate buffer of pH 7.5 at 40 °C the rate-determining step of the hydrolysis of aspirin is a water

Fig. 1 Hydrolysis scheme of aspirin at neutral pH in aqueous environment



attack assisted by the carboxylate group (Fig. 1). The intermediate is then cleaved in a fast reaction to form the end products, salicylic acid and acetic acid [7]. The overall reaction is temperature-sensitive following the Arrhenius equation. Two types of samples (A: 1 mM aspirin alone, B: 1 mM aspirin plus a gold NP suspension with a total amount of gold of 40 mg which translates roughly to 10^{17} NP in a final volume of 0.6 mL) were treated with two hydrolysis conditions (1: without, 2: with an alternating magnetic field, resulting in four combinations: A1, A2, B1 and B2). The alternating magnetic field is technically realized as a magnetic gradient, a typical equipment of all modern NMR systems (400 MHz Bruker Avance spectrometer, alternating gradient frequency = 3 kHz, gradient amplitude $\pm 10\%$ from maximal 55 Gauss/cm). The hydrolysis was directly measured by NMR using the averaged integrals of two proton resonances of the intact aspirin: the well resolved aromatic proton in the ortho position to the carboxylic acid, and the methyl group resonance (as an example see Fig. 2). The initial integral was set to 100% so that the decreasing integrals indicate the hydrolysis. The sample with aspirin plus gold NP showed a significantly increased hydrolysis if the alternating magnetic field was switched on (blue versus red line in Fig. 3). In contrast, for the sample without gold NPs the alternating magnetic field had no significant influence (black versus green line in Fig. 3). The orientation of the NP by the strong, but static magnetic field cannot be responsible since the experimental results clearly show no significant effect when aspirin was incubated with the gold NP suspension but without alternating magnetic field (red line in Fig. 3). Due to the instability of the gold NP samples, the

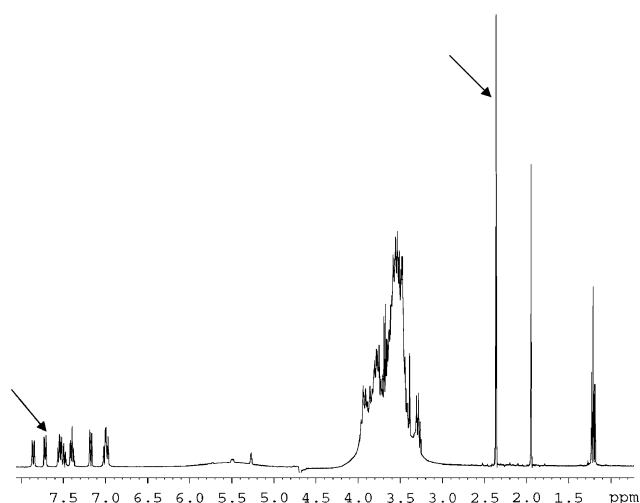


Fig. 2 Proton-Spectrum of a 1 mM solution of aspirin with Aurovist™ gold NPs with watergate water suppression; arrows indicate the resonances used for quantification

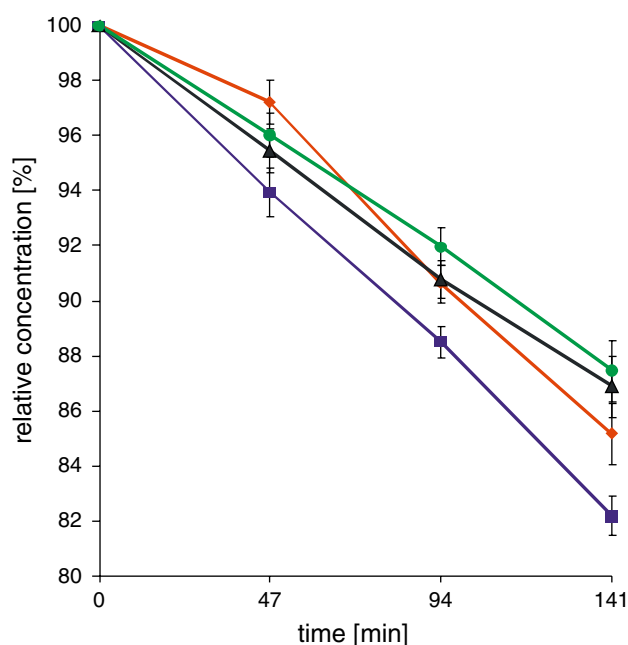


Fig. 3 Hydrolysis of aspirin. In blue: gold NPs (40 mg) plus alternating magnetic field. In red: gold NPs without alternating magnetic field. In black: with alternating magnetic field. In green: without alternating magnetic field. In all experiments a 1 mM concentration of aspirin was used. Error bars indicate standard deviation

observations were stopped after 141 min. After this period of time, the hydrolysis was accelerated from the averaged level of 86.5% calculated from the three control experiments (A1, A2, and B1) to a level of 82.2% for the experiment with gold NP and an alternating magnetic field (B2).

In the following, an explanation of the effect of stimulating the hydrolysis of aspirin is given. The gold NP used in this study with a gold core diameter of 1.9 nm and a small size distribution are covered by sulphur-bonded carboxylic acids to stabilize the NP. Recent results showed that gold NP, especially those stabilized by sulphur ligands, possess a magnetic moment [8]. This type of magnetism might be classified as superparamagnetism [9]. It can be rationalized by the formation of electron holes in the 5d-orbitals due to the gold–sulphur bond. The occurrence of these partially filled d-orbitals then gives rise to a magnetic moment similarly to the well known 3d-type ferromagnetics such as iron. Experimental data showed magnetic moments per Au atom attached to a sulphur-ligand from 0.006 [10] to 0.0093 μ_B [11].

The magnetic moment of gold NP depends highly on the geometry and bonding, and since only recently the structure of a gold NP with sulphur ligands could be analyzed [12], reliable predictions about magnetic properties can at present hardly be made. Assuming that the gold NP of this study exhibit a similar magnetic behaviour, the ratio of 0.6 between surface atoms and total atoms would lead to a magnetic moment of 0.004 μ_B averaged over all atoms of such a gold NP (roughly 150 gold atoms). This would amount to a magnetic moment of 0.6 μ_B for the whole gold NP which is comparable to the magnetic moment of a ferromagnetic iron atom (2.2 μ_B).

Having identified the exceptional magnetic properties of the gold NPs as responsible for the interaction with the alternating magnetic field, the question about the mechanism arises. In principle, in the case of the gold NP Aurovist™ different mechanisms to transfer the magnetic energy from the alternating magnetic field into the solution might operate.

- (i) Heating via hysteresis: Ferromagnetic NP of sizes above 100 nm show hysteretic behaviour but particles smaller than 10 nm are single-domain structured, do not show hysteresis, but transfer energy by relaxation processes [13].
- (ii) Heating via inductive coupling: This mechanism was proposed by Hamad-Schifferli et al. [14] but in the case of gold NP cannot explain the energy coupling. Consequently, this explanation has not been given again by this group in further work.
- (iii) Heating via relaxation: The dominant relaxation process depends on the anisotropy energy, the volume of the particle, viscosity of the solution and temperature. The very high anisotropy energy values of 10^9 J/m³ measured for gold NP [15] would favour Brown relaxation where the whole particle changes its orientation [16]. However, the small size (gold core diameter of 1.9 nm plus stabilizing ligand shell for the

Aurovist™ gold NP) would favour Néel relaxation [17] with a changing magnetic moment responsible for the energy conversion from magnetic to thermal [13].

In the liquid suspension, energy can also be transferred via frictional losses due to the magnetic torque produced by the alternating magnetic field and the remanent magnetization [18]. Without further information, it is impossible to specify which mechanism and/or mixture of mechanisms is operating. Assuming the Brown relaxation mechanism ferromagnetic (within this size regime: superparamagnetic) iron oxides produced thermal powers of ca. 100 W per gram [18]. In these cases a strong increase of temperature is obtained which was not observed in the present approach with gold NP. The relaxational power loss equation contains the squared magnetic moment of the particles [18]. Assuming a 100 fold reduced magnetic moment of the gold NP compared to an iron oxide NP, this would lead to a 10⁴ fold reduction of the power deposited (10 mW per gram). With this little amount of energy, only a small fraction of the solution can represent an activated/heated volume for the hydrolysis. Essentially, assuming Brown relaxation the rotational energy of the whole particle is transformed into rotational, vibrational and translational energy [19] of the surrounding nanoscopic layer of bulk solvent and aspirin molecules, thereby increasing the hydrolysis rate. This reasonable assumption can explain the overall increase in the hydrolysis rate without simultaneous bulk heating. However, typical hyperthermia conditions could probably also be used to hydrolyse pro-drugs as exemplified in this study.

Conclusions

The application of time and spatially resolved magnetic fields was successfully used to accelerate a typical activating reaction used for pro-drugs. Furthermore, the combined approach allows (i) full chemical flexibility in pro-drug design exploiting the vast chemical and medicinal experience in this field, (ii) application of the rich gold chemistry [20, 21] (iii) the direct observation by NMR, (iv) full control of the process with conventional NMR systems and (v) small amounts of deposited energy minimizing thermal side reactions. Pro-drugs already in use can now be tested with an appropriate nanoparticle-magnet system in conventional MRI instruments.

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