Note Added in Proof. Three relevant papers appeared after this manuscript was submitted.

Cruickshank et al.[53] reported the 104.1-MHz ^{27}Al MAS NMR spectra of barium aluminum glycolate and andalusite. The spectrum of the glycolate exhibits a signal maximum at δ 35.3 (and, as in our sample, a signal for octahedral aluminum from an impurity). The results for the two samples of barium aluminum glycolate at the three field strengths suggest that the 5-coordinate aluminum has a qcc $\simeq$ 4 MHz. The stongest signal in the 104.1-MHz spectrum of andalusite is at about δ 15. The quadrupole-induced upfield shift of the center of gravity for the 5-coordinate aluminum signal is about 8 ppm greater at 104.1 MHz than at 130.3 MHz [i.e., $(130.3/104.1)^2(-14.3 \text{ ppm}) = -22.4$ ppm], which in light of our Figure 6 leaves no doubt that the signal reported by Cruickshank et al.[53] near δ 15 results from 5-coordinate aluminum, not from octahedral aluminum as is indicated in their figure. (The octahedral aluminum gives an undetectably broad, far upfield signal at lower field strengths.[54] A much shorter pulse than the 10-μs pulse used[53] would appear desirable for recording ^{27}Al NMR spectra of solids, particularly when the quadrupole interactions are so large.[55,56]) The signal in their spectrum near δ 50 clearly results from a spinning sideband—the spinning rate of 3.7 kHz results in sidebands at 35-ppm intervals—not from the 5-coordinate aluminum centerband as is indicated in their[53] figure.

Our interpretation of the spectrum of andalusite is consistent with those recently reported by two other groups.[54,57] Lippmaa et al.[54] reported that the 130.4-MHz ^{27}Al MAS NMR spectrum of andalusite gives a value of δ 36.0 for the chemical shift of the 5-coordinate aluminum after correcting for the quadrupole-induced upfield shift. (We estimated δ 35.) Dupree et al.[57] reported the 104-MHz ^{27}Al MAS NMR spectra of two amorphous alumina films. In addition to signals for tetrahedral and octahedral aluminum, each film gives a signal near δ 28 that does not result from spinning sidebands. This signal is attributed to AlO_5 species because andalusite is reported[57] to give a signal for 5-coordinate aluminum at about δ 35.

Registry No. Barium aluminum glycolate, 97867-12-4; andalusite, 12183-80-1.

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Simplification of Two-Dimensional ^{1}H NMR Spectra Using an X-Filter[1]

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Abstract: X-Filtering is a new method for simplifying two-dimensional (2D) homonuclear ^{1}H NMR spectra, which is based on spin–spin couplings between protons and a NMR-active X-nucleus. The X-filtered 2D NMR spectra contain exclusively cross peaks and diagonal peaks from protons coupled to X. The procedure is applied to ^{1}H 2D correlated spectroscopy (COSY) and ^{1}H 2D nuclear Overhauser and exchange spectroscopy (NOESY). Potential applications include studies of small molecules as well as biological macromolecules which contain NMR-active X-nuclei. For example, with the use of X-filtering the coordination sites of NMR-active metal ions may be identified, which could otherwise often only be achieved with laborious chemical isotope labeling procedures.

The advent of two-dimensional NMR spectroscopy has revolutionized the analysis of homonuclear and heteronuclear spin systems in solution, both for small molecules as well as macromolecules such as proteins and nucleic acids.[2,3] Work with macromolecules benefits in particular from techniques capable of simplifying 2D NMR spectra and thus enhances the accessible information content. Examples are multiple quantum spin filters,[4] X-relayed magnetization transfer,[5] and editing techniques based on gated heteronuclear decoupling.[6] The present paper introduces a novel filtering procedure, which utilizes heteronuclear spin–spin couplings to simplify homonuclear ^{1}H 2D NMR spectra. This technique also modifies in characteristic ways the multiplet structure of cross peaks connecting protons which are coupled to a particular X-nucleus. The 2D NMR measurements needed for this method ensure that both the complete *and* the X-filtered spectrum are obtained from one experimental setup, without an increase in measuring time relative to the corresponding conventional ^{1}H 2D NMR experiment. As an illustration, ^{113}Cd-

(1) Abbreviations used: NMR, nuclear magnetic resonance; rf, radio frequency; FID, free induction decay; 1D, one-dimensional; 2D, two-dimensional; ppm, parts per million; *J*, spin–spin coupling constant; COSY, two-dimensional correlated spectroscopy; MQF, multiple quantum filter; 2QF-COSY, two quantum filtered COSY; NOE, nuclear Overhauser enhancement; NOESY, two-dimensional nuclear Overhauser and exchange spectroscopy; MQC, multiple quantum coherence; EDTA, ethylenediaminetetraacetic acid; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; TSP, trimethylsilylpropionic acid sodium salt.
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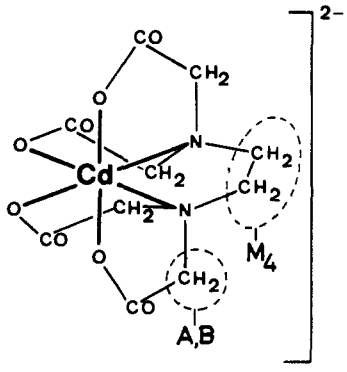

Figure 1. Schematic drawing of the hexacoordinated form of the Cd^{2+}–EDTA complex. Coordinative bonds are indicated with heavy lines. (For background information see Jensen et al.[8]) The broken circle contains one of four equivalent proton AB spin systems; the broken elipse contains a proton M_4 spin system.

filtered ^1H COSY, ^1H 2QF-COSY, and ^1H NOESY spectra of a solution containing the $^{113}Cd^{2+}$–EDTA complex (Figure 1) and alanine in ^2H_2O are presented and analyzed in terms of the product operator formalism.[7]

Results

Figure 2A shows the ^1H NMR spectrum of the mixed solution of $^{113}Cd^{2+}$–EDTA and alanine, which we used as the test sample. The assignments are indicated in the figure. The protons of the four equivalent acetate methylene groups in $^{113}Cd^{2+}$–EDTA (Figure 1) give rise to an AB ^1H NMR pattern, which is further split owing to coupling with ^{113}Cd. The M_4 spin system resonance of the ethylene bridge of EDTA (Figure 1) is also split into a doublet by the ^{113}Cd spin. The other signals belong to alanine and to the Tris buffer, as indicated in Figure 2A. (Tris gives rise to two singlet lines of unequal intensity, which must represent two different forms of the molecule, possibly free and bound to Cd^{2+}–EDTA. Exchange between the two forms is manifested by cross peaks in NOESY; see Figure 4B.) The $^{113}Cd^{2+}$–EDTA complex was chosen as the model system for the present paper because, even though it exhibits spectral features typically encountered also in macromolecules, its spin systems are rather simple. In the following we refer to the acetate CH_2 protons as spins I_A and I_B, the ethylene bridge protons as spins I_M, and the ^{113}Cd nucleus as spin S. The spin–spin coupling constants are denoted J_{AB}, J_{AS}, J_{BS}, and J_{MS}.

A. X-Filtered Two-Dimensional Correlated ^1H NMR Spectroscopy (X-Filtered COSY or 2QF-COSY). Figure 2B shows a contour plot presentation for conventional phase-sensitive COSY of the test sample, which was recorded with the pulse sequence $90°_x$–t_1 – $90°_x$–t_2. The spectrum is phased to yield absorptive lines for cross peaks and dispersive lines for diagonal peaks. This is most clearly seen for the spectrum of Ala. As a consequence of the spin–spin couplings with ^{113}Cd, EDTA gives rise to peculiar cross peak and diagonal peak multiplet patterns.[9] To achieve *X-filtration* the normal COSY pulse sequence has to be extended with a π pulse on X (Figure 3A). This rf pulse on X is applied in every second scan, and the FID is stored in a different computer memory location. X-Filtered COSY (Figure 2C) results from subtraction of the FID with X-pulse from the FID without X-pulse. All resonances that are not coupled with X are suppressed, and the fine structure of the remaining peaks is changed as a result of the modified pulse sequence. All the cross peaks observed in X-filtered ^1H COSY connect protons which are coupled to the same heteronucleus X. The same X-filter can also be applied with proton MQF-COSY. Figure 2D shows a contour plot of a X-filtered 2QF-COSY experiment. Only protons coupled to the heteronucleus and to at least one further nonequivalent proton are visible. Therefore, the diagonal peak due to the M_4 spin system of the ethylene protons observed in Figure 2C is not present in spectrum D. The I_AI_B diagonal peaks show the same phases as the corresponding cross peaks, just as in a normal 2QF-COSY.[4c]

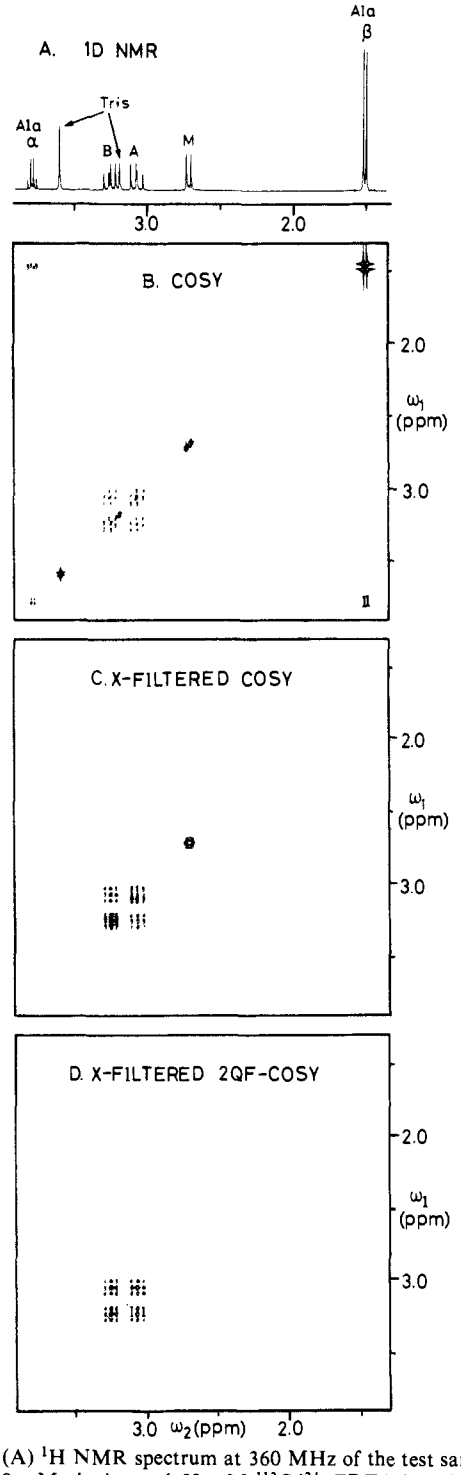

Figure 2. (A) ^1H NMR spectrum at 360 MHz of the test sample containing 200 mM alanine and 50 mM $^{113}Cd^{2+}$–EDTA in Tris-buffered ^2H_2O at p^{2}H 7.0, 25 °C. The resonances of the AB spin system of the acetate methylene group of EDTA (Figure 1) are doubled by the coupling with ^{113}Cd ($I = {}^1/_2$, isotope enrichment 95%). Similarly, the M_4 singlet (Figure 1) is split into a doublet by the ^{113}Cd. Additional resonance lines from alanine and from the Tris buffer are also identified. Chemical shifts are referred to external TSP. (B–D) Contour plots of phase-sensitive ^1H COSY spectra of the test sample at 360 MHz and 25 °C. No distinction is made between positive and negative intensities: (B) conventional COSY; (C) X-filtered COSY. Only protons coupled to the ^{113}Cd can be observed. (D) X-Filtered 2QF-COSY. Only protons coupled to ^{113}Cd and to at least one nonequivalent proton can be observed.

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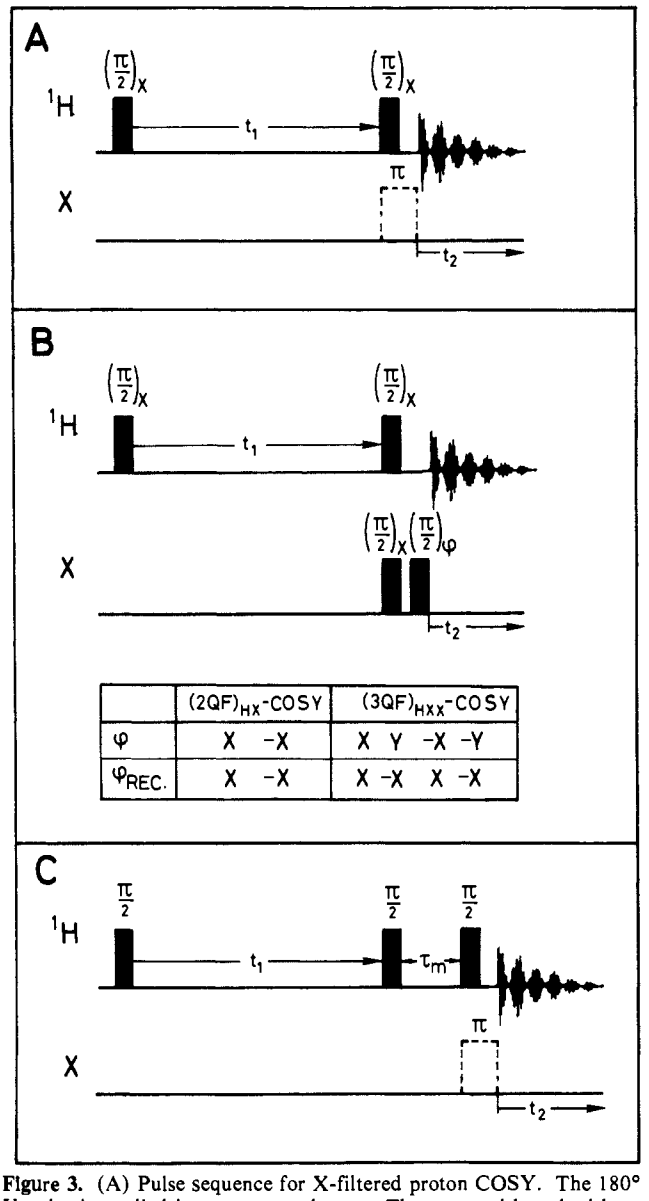

Figure 3. (A) Pulse sequence for X-filtered proton COSY. The 180° X-pulse is applied in every second scan. The scans with and without X-pulse are stored separately, so that the two subfiles can be arbitrarily combined. The difference of the two subfiles corresponds to X-filtered COSY (Figure 2C). (B) Proposed phase cycling schemes for a heteronuclear two-quantum filter $(QF)_{HX}$ with proton detection (this is equivalent to the pulse scheme of Figure 3A combined with subtraction of the two subfiles) and a heteronuclear three-quantum filter $(3QF)_{HXX}$ with proton detection. The subscripts indicate the type of spins participating in the selected heteronuclear multiple quantum coherence. (C) Pulse sequence for X-filtered proton NOESY. The 180° X-pulse is applied in every second scan. The scans with and without X-pulse are stored separately so that the two subfiles can be arbitrarily combined. The difference of the two subfiles corresponds to X-filtered NOESY (Figure 4C).

B. X-Filtered Two-Dimensional Nuclear Overhauser and Exchange Spectroscopy (X-Filtered NOESY). The contour plot of a conventional NOESY[10] spectrum of the test sample recorded with the pulse sequence $90°-t_1-90°-\tau_m-90°-t_2$ (Figure 4B) contains cross peaks between αH and βCH_3 of Ala, between different protons of $^{113}Cd^{2+}$–EDTA, and between the two Tris resonances. Protons coupled to the X-nucleus give rise to peculiar cross-peak multiplet patterns, which are reminiscent of the observations in COSY (Figure 2B and ref 9). The NOESY peaks

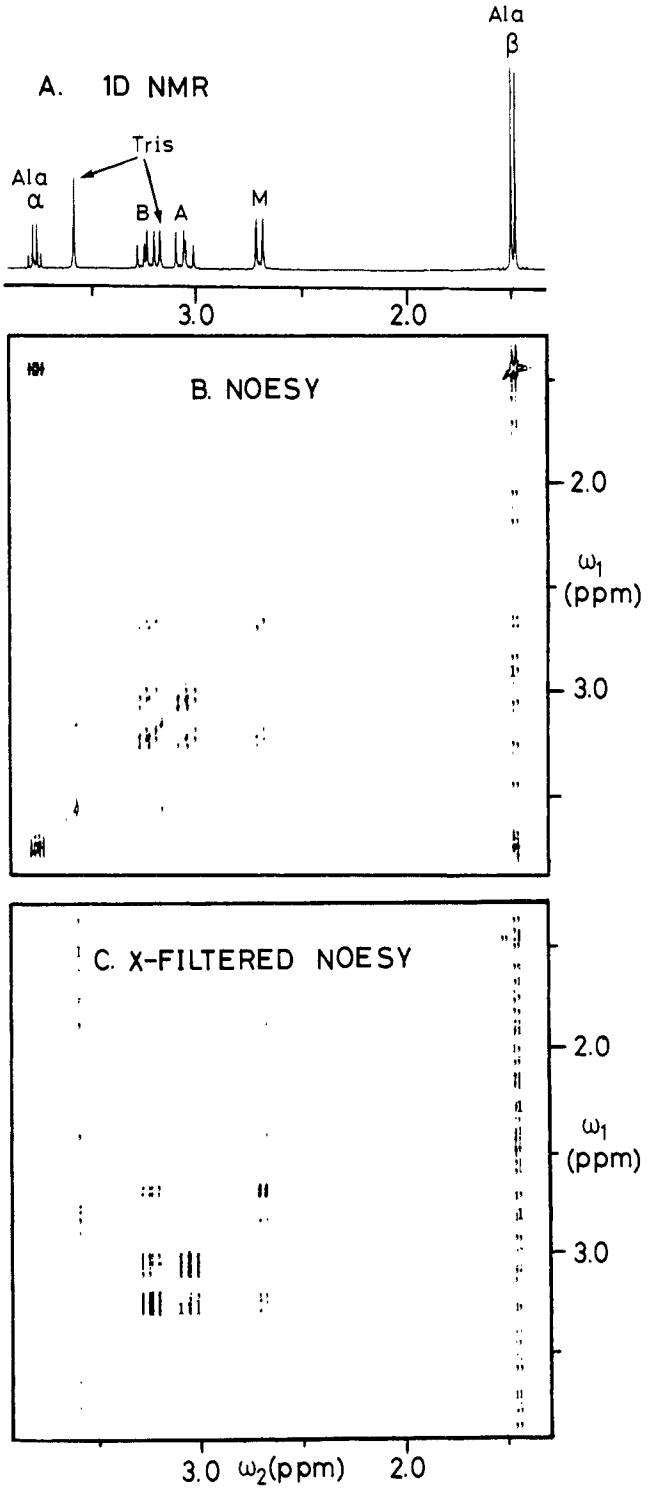

Figure 4. Contour plots of phase-sensitive proton NOESY spectra of the test sample containing $^{113}Cd^{2+}$–EDTA, alanine, and Tris buffer at 360 MHz and 25 °C recorded with a mixing time of 0.8 s. No distinction is made between positive and negative intensities. (A) Same 1D NMR spectrum as in Figure 2A. (B) Conventional NOESY. All NOE's are positive. (C) X-Filtered NOESY corresponding to the difference of the two subfiles recorded with the pulse sequence of Figure 3C. Only protons coupled to ^{113}Cd are observed. The low-field proton of the acetate CH_2 group of EDTA (I_B) and the ethylene bridge (I_M) (see Figure 1) are connected by a NOE. Spurious signals along ω_1 at the ω_2 positions of Ala βCH_3 and the lower field line of Tris represent t_1 noise.

corresponding to protons which are not coupled with X can be suppressed by insertion of a 180° X-pulse in every second scan together with the detection pulse and subtraction of the FID's with and without X-pulse (Figure 3C). Figure 4C shows a contour plot of a X-filtered NOESY experiment. The spectrum consists

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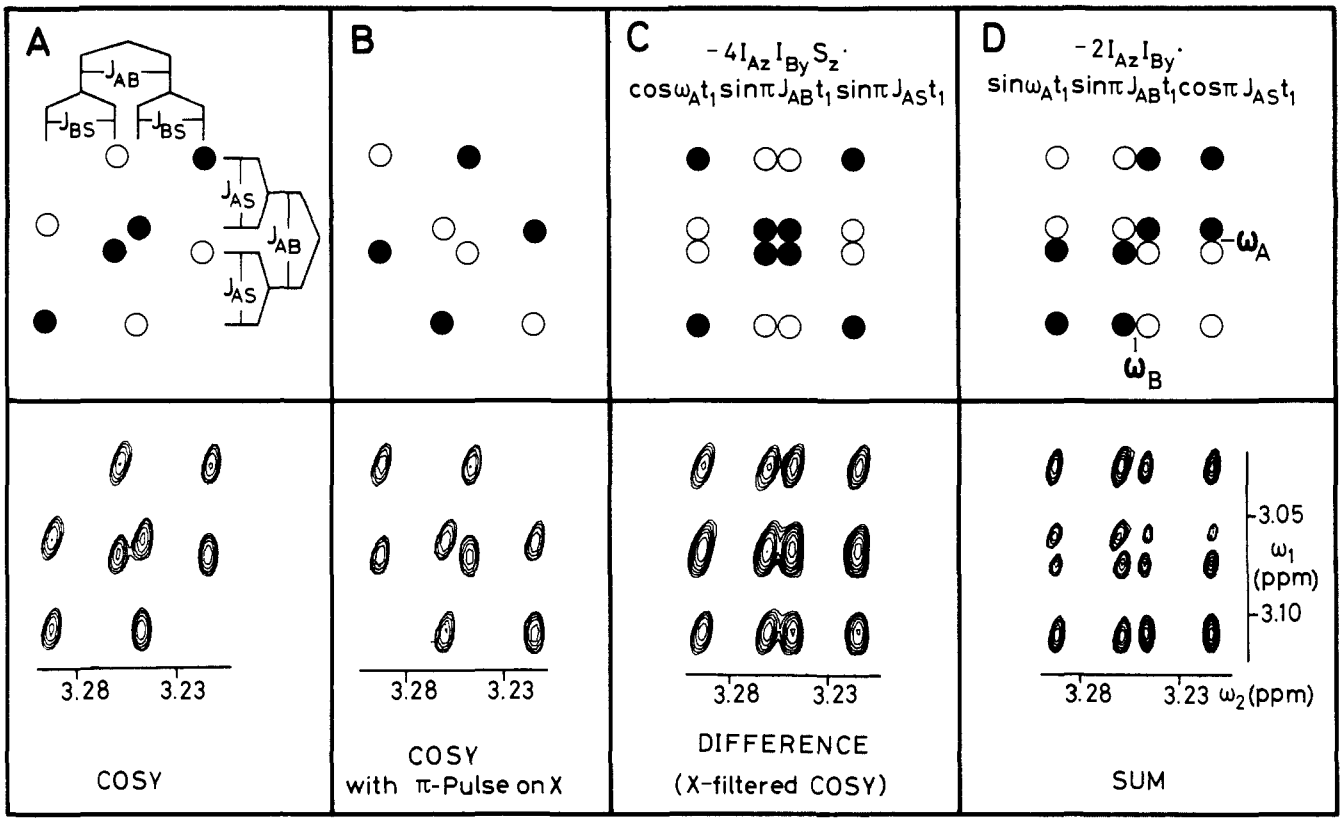

Figure 5. Comparison of the experimental multiplet pattern of the COSY cross peak connecting the spins I_A and I_B of the acetate methylene protons in $^{113}Cd^{2+}$–EDTA (Figure 1) with the pattern predicted for an I_AI_BS spin system with $|J_{AB}| = 16.5$ Hz, $|J_{AS}| = 13.6$ Hz, and $|J_{BS}| = 12.0$ Hz. In the predicted patterns full and open circles indicate opposite signs of the multiplet components. In the experimental spectra positive and negative components are not distinguished, but they coincide in all instances with the predicted signs. (A) COSY. The analysis in terms of spin–spin coupling constants is indicated. (B) Modified ^1H COSY, with a 180° pulse on ^{113}Cd applied simultaneously with the ^1H mixing pulse. (C) X-Filtered ^1H COSY obtained by subtraction of A and B. In the experimental spectrum the fine structure along ω_1 is not fully resolved. The second product operator term of eq 2, which describes this spectrum, is also indicated. (D) Multiplet pattern obtained by addition of A and B, which is represented by the first product operator term of eq 2.

exclusively of cross speaks and diagonal peaks of protons with a resolved coupling to the ^{113}Cd ion. (In molecules containing more than one X-nucleus one could in principle also observe NOE cross peaks between protons coupled to different heteronuclei. This, however, would demand a simultaneous cross relaxation between these two protons *and* between the two X-nuclei.[12]) All peaks in the spectra 4B and 4C are in the absorption mode.

Discussion

In the phenomenological descriptions of X-filtered ^1H COSY and X-filtered ^1H NOESY presented in the preceding section, it was pointed out that protons coupled with X give rise to peculiar multiplet fine structures. For improved clarity, one of the cross peaks connecting the spins A and B in $^{113}Cd^{2+}$–EDTA (Figure 1) is shown on an enlarged scale in Figure 5 (COSY) and 6 (NOESY). At a first glance the experimental multiplet patterns in COSY and NOESY appear rather similar. However, it is also apparent that there are fundamental differences between the two experiments, which lead to a different dependency of the experimental peak shapes and intensities on the coupling constants. These intricate details of the presently proposed techniques are further analyzed below; also included is a product operator treatment.[7]

Fundamentally, X-filtering relies on the fact that the X spins are not affected by the rf pulses at the ^1H frequency used for the ^1H COSY or ^1H NOESY. In particular, since the X spins are not affected by the ^1H observation pulse, only proton transitions occur for which the X spin has the same polarization during evolution and detection. This leads to the forementioned peculiar cross peak patterns (Figure 5A and 6A), which were previously also observed in ^{113}Cd-metallothionein-2.[9] In modified ^1H COSY or ^1H NOESY experiments, where a 180° pulse at the X-frequency is applied simultaneously with the ^1H observation pulse, those transitions occur which have opposite X-spin polarization during t_1 (before the 180° X-pulse) and t_2 (after the 180° X-pulse). Starting with the multiplets in Figure 5A and 6A, this results in a left shift by $|J_{BS}|$ of the four-component square array in the upper right, and a right shift by $|J_{BS}|$ of the four components in the lower left.

In conventional COSY with the pulse sequence $90°_x$–t_1–$90°_x$–t_2, the part of the density operator which describes a cross peak between two coupled protons A and B is given by[7]

$$\sigma(t_2=0) = -2I_{Az}I_{By} \sin(\omega_A t_1) \sin(\pi J_{AB} t_1) \quad (1)$$

Equation 1 describes the magnetization obtained immediately after the second 90° pulse (Figure 3A). The term $2I_{Az}I_{By}$ gives rise, after Fourier transformation, to an antiphase doublet structure along ω_2. The product of the two sine terms in eq 1 gives rise to an antiphase doublet structure along ω_1 of the cross peak at the position ($\omega_1 = \omega_A$ and $\omega_2 = \omega_B$).[11]

To allow for the coupling of both I_A and I_B to S in $^{113}Cd^{2+}$–EDTA, an additional $\cos(\pi J_{AS} t_1)$ modulation during the evolution period t_1 must be added to eq 1, and a new term modulated with $\sin(\pi J_{AS} t_1)$ contributes to the cross peaks. The part of the density operator which yields the AB cross peak in an I_AI_BS spin system where both I_A and I_B are coupled to S, is then given by

$$\sigma(t_2=0) = -2I_{Az}I_{By} \sin(\omega_A t_1) \sin(\pi J_{AB} t_1) \cos(\pi J_{AS} t_1) - 4I_{Az}I_{By}S_z \cos(\omega_A t_1) \sin(\pi J_{AB} t_1) \sin(\pi J_{AS} t_1) \quad (2)$$

The second term of eq 2 can only be observed if $J_{BS} \neq 0$, since otherwise it could not refocus to observable in-phase magnetization during t_2. The first term is antiphase with respect to J_{AB} and in-phase with respect to J_{AS}, whereas the second term is antiphase

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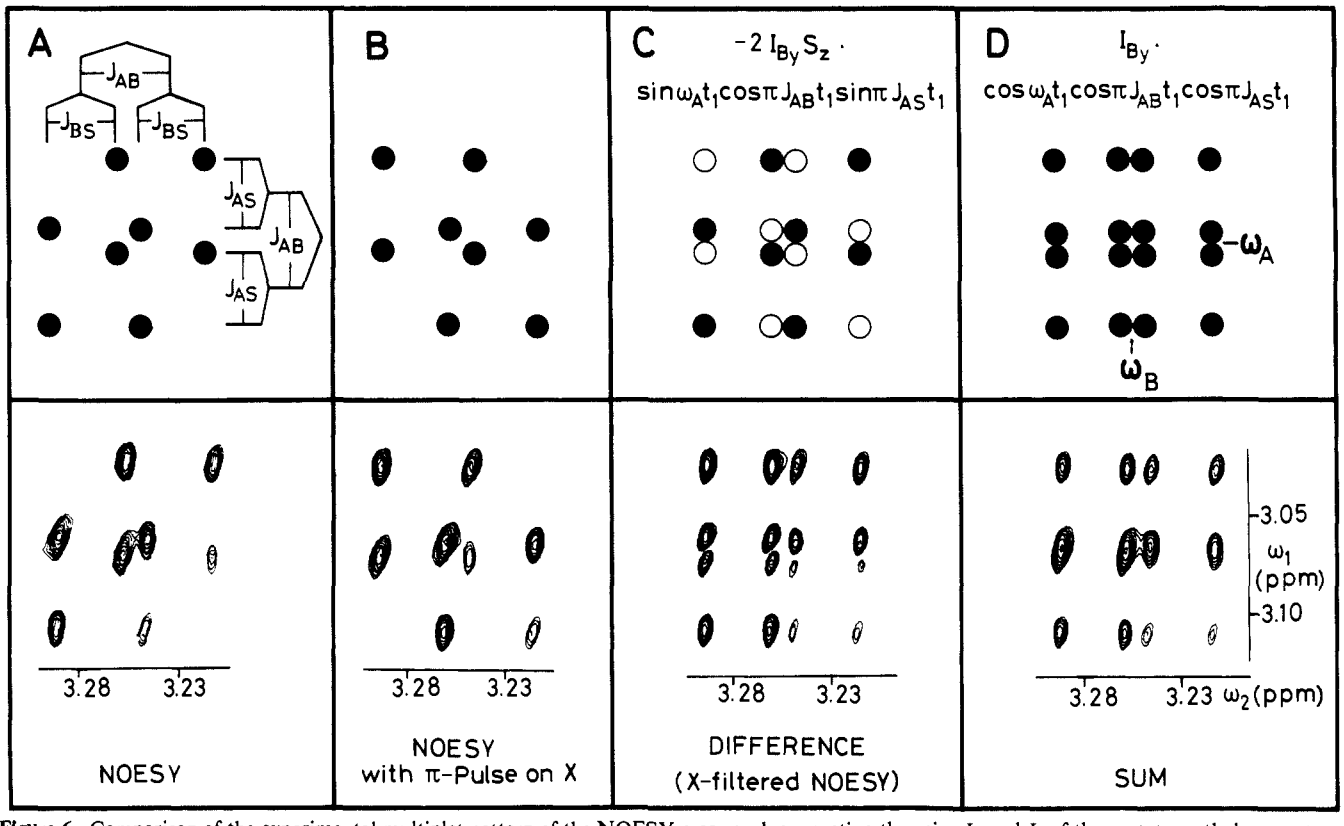

Figure 6. Comparison of the experimental multiplet pattern of the NOESY cross peak connecting the spins I_A and I_B of the acetate methylene protons in $^{113}Cd^{2+}$-EDTA (Figure 1) with the pattern predicted for an I_AI_BS spin system with $|J_{AB}| = 16.5$, $|J_{AS}| = 13.6$, and $|J_{BS}| = 12.0$ Hz. In the predicted spectrum full and open circles indicate opposite signs of the multiplet components. In the experimental spectrum positive and negative components are not distinguished. (A) ^1H NOESY. The analysis in terms of spin–spin coupling constants is indicated. (B) Modified ^1H NOESY with a 180° pulse on ^{113}Cd applied simultaneously with the ^1H observation pulse. (C) X-Filtered NOESY obtained by subtraction of A and B. In the experiment the signs of the peaks coincide with those predicted, but they are not distinguished in this presentation. The cross peak is represented by the second product operator term of eq 4. (D) Multiplet pattern obtained by addition of the experimental spectra A and B, which is represented by the first product operator term of eq 4. In the experiment the fine structure along ω_1 is not fully resolved.

to both, the homonuclear and the heteronuclear coupling. In normal ^1H COSY the superposition of these two terms leads to the multiplet pattern of Figure 5A for the AB cross peak of an I_AI_BS spin system.

These considerations form the background for the X-filter procedure with COSY. A 180° pulse on X applied simultaneously with the proton mixing pulse in every second scan (Figure 3A) inverts the sign of the product operator terms containing an S_z contribution, such as the second term of eq 2, whereas all other terms are invariant under this operation. Addition or subtraction of the FID's with and without X-pulse then yield two complementary 2D NMR spectra. In the difference spectrum, which corresponds to the *X-filtered* spectrum, there remain only diagonal peaks of protons with a nonvanishing heteronuclear coupling, and cross peaks between coupled protons which are both also coupled to the *same* X-nucleus (Figure 2C). One AB cross peak is plotted on an enlarged scale in Figure 5C. It corresponds to the second term of eq 2, i.e., to the product operator representing antiphase magnetization to both the heteronucleus S and the coupled proton I_A. The sum spectrum, however, contains all cross peaks and diagonal peaks of normal COSY (no overview plot is shown), but the multiplet fine structure of peaks due to heteronuclear coupled protons has changed as a result of losing the term $4I_{Az}I_{By}S_z$ when adding the FID's with and without X-pulse. Therefore, the cross peak corresponds to the first term of eq 2, i.e., the product operator representing in-phase magnetization with respect to the heteronucleus S and antiphase magnetization with respect to the coupled proton I_A (Figure 5D). Note that the full array of 16 multiplet components represented by each of the two product operators of eq 2 (Figure 5 C and D) can only be generated by linear combinations of the two ^1H COSY experiments with and without X-pulse, which yield two complementary sets of eight multiplet components (Figure 5A,B).

Formally, the X-filter can be regarded as a heteronuclear two-quantum filter with proton detection. If the 180° X-pulse is split into two 90° pulses (Figure 3B), the first pulse generates heteronuclear two-quantum coherence by transforming $-4I_{Az}I_{By}S_z$ into $+4I_{Az}I_{By}S_y$. The second pulse combined with appropriate receiver cycling (table in Figure 3B) acts as a heteronuclear two-quantum filter. The concept of heteronuclear multiple quantum filters with proton detection can then be extended to more complicated spin systems. For example, in cases where two protons I_i and I_j are coupled to *two* NMR active X-nuclei S_k and S_l, product operator terms of the general form $I_{iz}I_{jy}S_{kz}S_{lz}$ contribute to COSY cross-peak patterns. With a phase cycle which selects for three-quantum coherence between S_k, S_l, and one I spin (an example is given in the table of Figure 3B), terms of the types $I_{iz}I_{jy}$, $I_{iz}I_{jy}S_{kz}$, and $I_{iz}I_{jy}S_{lz}$ are suppressed and the homonuclear 2D spectrum contains only signals of protons which are coupled to at least two X-nuclei. As a notation for heteronuclear spin filters, we propose to use subscripts indicating the spins actively involved in the selected multiple quantum coherence (Figure 3B).

Similar arguments to those used for rationalizing X-filtered ^1H COSY apply for X-filtered ^1H NOESY. The part of the density operator which represents a cross peak between two protons I_A and I_B in a NOESY experiment, $90°-t_1-90°-\tau_m-90°-t_2$, is at the beginning of the detection period t_2 given by

$$\sigma(t_2=0) = I_{By} \cos(\omega_A t_1) \cos(\pi J_{AB} t_1) \quad (3)$$

Two-dimensional Fourier transformation yields a cross peak at the position ($\omega_1 = \omega_A$ and $\omega_2 = \omega_B$). The evolution of I_{By} during t_2 is responsible for the in-phase doublet fine structure along ω_2, and the trigonometric functions in eq 3 are responsible for the in-phase doublet fine structure along ω_1. To account for the couplings of I_A and I_B to a heterospin S, two extensions have to be made: The product operator term $I_{By} \cos(\omega_A t_1) \cos(\pi J_{AB} t_1)$

of eq 3 is further modulated with cos ($\pi J_{AS}t_1$), and an additional term $-2I_{By}S_z$ sin ($\omega_A t_1$) cos ($\pi J_{AB}t_1$) sin ($\pi J_{AS}t_1$) contributes to the cross peak. This second term arises from magnetization $2I_{Ay}S_z$ at the end of t_1, which is converted to heteronuclear longitudinal two-spin order $2I_{Az}S_z$ by the second ^1H 90° pulse. During τ_m this two-spin order may be transferred to $2I_{Bz}S_z$ by exchange or by cross relaxation,[12] which is converted to $-2I_{By}S_z$ by the third 90° pulse. The parts of the density operator that contribute to the cross peak thus are

$$\sigma(t_2=0) = I_{By} \cos(\omega_A t_1) \cos(\pi J_{AB}t_1) \cos(\pi J_{AS}t_1) - 2I_{By}S_z \sin(\omega_A t_1) \cos(\pi J_{AB}t_1) \sin(\pi J_{AS}t_1) \quad (4)$$

Two-dimensional, phase-sensitive Fourier transformation leads to multiplet patterns of the diagonal peaks and the cross peaks which are similar to the corresponding COSY patterns, except that the signs and intensities of the multiplet components are different (compare Figure 5A and 6A). The antiphase term $2I_{By}S_z$ in eq 4 is only detectable if $J_{BS} \neq 0$. In a modification of ^1H NOESY, a 180° pulse on X applied simultaneously with the ^1H observation pulse (Figure 3C) inverts the sign of the product operator terms containing S_z. Subtraction of the FID's with and without X-pulse yields the X-filtered ^1H NOESY spectrum, which contains only cross peaks connecting protons which are *both coupled to the X-spin* (Figures 4C and 6C). The sum of the FID's recorded with and without X-pulse contains all peaks found in ^1H NOESY, but with multiplet patterns containing, in addition, components corresponding to inverted X-spin polarizations during t_2. Note again that the full array of 16 multiplet components described by each of the product operator terms of eq 4 can only be obtained by linear combinations of the two ^1H NOESY experiments with and without 180° pulse on X.

The absolute intensity of the sum of the fine structure components in the diagonal peaks and cross peaks of X-filtered ^1H COSY or X-filtered ^1H NOESY is the same as in the corresponding normal ^1H experiments recorded with identical measuring time. However, this intensity is distributed among twice the number of multiplet components in the X-filtered experiments, so that the intensity of each individual multiplet component is reduced by a factor of 0.5 (Figures 5C and 6C). Depending on the size of the different couplings, positive or negative interference due to overlap between two or more multiplet components tends to be more pronounced in the X-filtered spectra than in the corresponding conventional experiments.

Experimental Section

A 50 mM solution of 95% enriched $^{113}Cd^{2+}$–EDTA in ^2H_2O containing Tris buffer at p^{2}H 7.0 was kindly provided by Dr. M. Vašák. Alanine (200 mM) was added for the test sample used. The NMR experiments were performed on a Bruker AM 360 spectrometer equipped with an Aspect 3000 computer and modified to allow ^1H observation at 360 MHz and ^{113}Cd pulsing at 80 MHz. All spectra were recorded with time-proportional phase incrementation (TPPI) in both dimensions, and the carrier was placed in the center of the spectrum.[13] To minimize t_1 ridges, the NOESY data were aquired with sine modulation.[14] The digital resolution was 0.6 Hz along ω_2 and 4.8 Hz along ω_1. For the cross-peak patterns shown in Figures 5 and 6, zero filling to two and four times the original size along ω_2 and along ω_1, respectively, was applied, while the overview plots shown in Figures 2 and 4 resulted with twofold zero-filling in ω_1 only. After multiplication with phase-shifted sine bell windows in the t_2 and t_1 directions, a phase-sensitive Fourier transformation was carried out in both dimensions. Instead of a 180° ^{113}Cd pulse, a composite $90°_x$–$240°_y$–$90°_x$ pulse sequence was used.[15] The two subfiles with and without X-pulse were recorded in a *scan interleaved* mode; i.e., a scan without the X-pulse was immediately followed by a scan with the X-pulse, and stored in separate computer locations: 4 scans per t_1 value and subfile were recorded for COSY and 2QF-COSY and 16 scans per t_1 value for NOESY. The individual scans were separated by a relaxation delay, so that the overall repetition time was 6 s.

Conclusions

The presently proposed method for the performance of X-filtered two-dimensional homonuclear proton NMR is particularly attractive for simplifying 2D NMR spectra of complex macromolecules containing NMR-active X-nuclei. For example, X-filters may replace chemical procedures of isotope exchange for identification of the binding partners of metals in proteins. X-filters should also be useful for enhancing the reliability of resonance assignments in small organic molecules or inorganic metal complexes, whenever heteronuclear coupled protons are located in crowded regions of the ^1H NMR spectra. The general versatility of the X-filtration procedure enables applications with COSY as well as NOESY experiments. It may further be useful in unravelling complex 2D multiple quantum spectra. In this context the X-filter can be considered as a high pass filter, i.e., a heteronuclear p quantum spin filter which is passed only by protons coupled to at least (p - 1) spins X.

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Registry No. $^{113}Cd^{2+}$-EDTA^{2-}, 103835-77-4; alanine, 56-41-7.

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