M) with rabbit uterine cytosol (containing 560 fmol/mg of proteins of PR) was recorded for the sample pressed into a 3-mm wafer after protamine sulfate precipitation,[24] with no prior dilution in an alkali-metal halide matrix.

Experiment 2. A 3-mm wafer was prepared under the same conditions as in experiment 1 except a 500-fold excess of the unlabeled steroid (RU 486) was added to the incubation medium (to saturate the receptor specific binding sites).

The subtraction of the FT-IR spectrum of the latter sample, in which the organometallic-labeled steroid present is nonspecifically bound, from that of the first sample yields a difference spectrum in which the carbonyl intensity is proportional to the extent of specific binding in the first sample. In order to compensate for the difference in thickness between the two sample wafers, the absorbances of the ν(OH) overtone of the protein at 6700 cm^{-1} in the two spectra were measured and a normalization factor N was calculated from the ratio of these intensities (eq 1) (A is the absorbance of a ν(CO) band.

$$A(\text{sp}) = A(\text{expt 1}) - N(A(\text{expt 2})) \quad (1)$$

The difference spectrum represented by eq 1 (Figure 2) shows clearly two of the bands characteristic of the carbonyl moiety of the cluster at 2025 and 2050 cm^{-1}, while the third and least intense signal at 2090 cm^{-1} is not clearly discerned above the background noise. The amount of organometallic cluster detected here is in the range of 1 pmol. In order to achieve the necessary sensitivity to observe these relatively weak signals, we employed an InSb detector, which is the most sensitive detector in the 2100-1900-cm^{-1} region available commercially. This set of experiments shows the feasibility of this new sensitive receptor assay of wide applicability in principle, together with a burgeoning area of application for transition-metal cluster complexes.

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Examination of an Alternate Structure for $Ba_2YCu_3O_{6+x}$ Using Single-Crystal X-ray Diffraction

Sir:

With the discovery that $Ba_2YCu_3O_{6+x}$ exhibits superconductivity at approximately 90 K, a great deal of work has been done to elucidate the crystal structure of this material. Neutron powder diffraction[1,2] and X-ray single-crystal diffraction[3,4] on both the orthorhombic and tetragonal forms of this material have proposed a layered variant of perovskite, with oxygen vacancies both in the Y layer and in the Cu layer between the Ba layers. In all this work, the cations have been ordered, with possibly some small interchange between the Y and Ba sites.[5] However, measurements using X-ray absorption near-edge structure (XANES)[6,7] have led to the proposal of an alternate structure, where Cu has substituted into the Y site at a level of 0.2-0.3 mole fraction. We have examined the single-crystal X-ray data taken on a number of tetragonal and orthorhombic crystals to determine if this model might be correct. Since the X-ray scattering factors for Cu and Y are very different (unlike the neutron scattering factors), the single-crystal X-ray structure should be sensitive to this substitution.

Table I. R Values Obtained for Cu Substitution into the Y Site

	mole fraction of Cu			
preparation	0.0	0.05	0.10	0.20
orthorhombic	0.0521	0.0529	0.0541	0.0548
tetragonal (700 °C, N_2)	0.0427	0.0467	0.0620	0.1198
tetragonal (melt grown)	0.0317	0.0318	0.0322	0.0321

Experimental Section. The crystals analyzed were prepared by standard techniques, and the analysis of their structures has appeared previously.[3,4] Briefly, single-crystal X-ray data were taken by using an automated Nicolet P3F diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). The materials were assumed to be modified perovskites, and therefore, atomic positions were assigned on the basis of this structure and refined by using the SHELXTL program package.[8] The major difference between the orthorhombic and tetragonal structures is that the Cu layer between the Ba layers contains partially occupied O sites in the orthorhombic material, while these sites are vacant in the tetragonal form.

There is nothing unusual about the Y sites in any of these crystals that would make one suspect substitution. Both the positional and thermal parameters are well-behaved. In order to test the model proposed from the XANES data, the structures of an orthorhombic crystal,[3] a tetragonal crystal prepared by annealing at 700 °C under nitrogen,[4] and a tetragonal crystal grown from a melt[9] were refined with the occupancy of the Y site at ($^1/_2$, $^1/_2$, $^1/_2$) fixed at various Y/Cu ratios. The site was always assumed to be fully occupied.

Results and Discussion. The R values obtained from each of the refinements are presented in Table I. The differences between the tetragonal and orthorhombic material are caused not by differences in the cation positions but by differences only in the oxygen positions. Therefore, one would assume that any disorder in the cation positions would manifest itself in both orthorhombic and tetragonal material. In all three cases examined, the R value increased as Cu was allowed to substitute into the Y site, even though the number of parameters being refined also increased. In addition, the thermal parameters for the Cu atom became very large. Therefore, if any substitution for Y by Cu is taking place, it is at a level much lower than the 0.2-0.3 mole fraction determined from the XANES data. We feel that the perovskite structure with the ordered cations proposed from both neutron and X-ray diffraction measurements is, therefore, correct and that there must be another explanation for the XANES data.

One should note that the reason for the proposed substitution of Cu in the Y site is the observation of a 2.5 Å Cu-O distance. We wish to point out that, in the accepted structure of the tetragonal material, we determined a Cu-O distance of 2.466 (13) Å between the Cu in the layers near Y and the O in the Ba layers.

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In the orthorhombic material, this distance is slightly shortened to 2.350 (14) Å. Recently published high-temperature neutron diffraction work[10] reports a 2.543 (11) Å Cu–O distance at 600 °C. This may account for the distance observed in the XANES data, especially if, during measurement using the intense synchrotron source, localized heating took place and converted some of the material to the tetragonal form.

The other possibility for the observation of a disorder may be related to this material's sensitivity to its thermal history. The sample used for the XANES work may have accidentally been prepared with an anomalous composition. However, on the basis of our data, any disorder between Y and Cu is not routinely observed in either ceramic or melt-grown material.

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^{43}Ca Nuclear Magnetic Resonance Spectra of Ca^{2+}–S100 Protein Solutions

Sir:

Dramatic increase of the line width of ^{43}Ca ($I = {}^{7}/_{2}$) NMR for Ca^{2+}–protein solution has hampered the detection of ^{43}Ca NMR for Ca^{2+}–protein (1:1 per binding site in the protein) complex by using a conventional NMR spectrometer equipped with conventional probe.[1,2]

S100 proteins are nerve specific proteins and belong to a family of EF-loop Ca^{2+}-binding proteins such as calmodulin or troponin C.[3-5] $S100_b$ protein and $S100_{a0}$ protein are homodimer proteins consisting of two α subunits (M_R = 10400) and of two β subunits (M_R = 10500), respectively, while $S100_a$ protein is a heterodimer protein consisting of one α and one β subunit.[3]

We show herein that ^{43}Ca NMR spectra of Ca^{2+}–$S100_b$ protein and Ca^{2+}–$S100_{a0}$ protein solutions consist of two distinguishable resonances that correspond to Ca^{2+} ions bound to different sites of the $S100_b$ and $S100_{a0}$ proteins. This is the first report of ^{43}Ca NMR signals ascribable to two discernible slowly exchanging Ca^{2+} ion binding sites.

$S100_b$, $S100_{a0}$, and $S100_a$ proteins were purified to gel-electrophoretical homogeneity by the method described previously.[6-10]

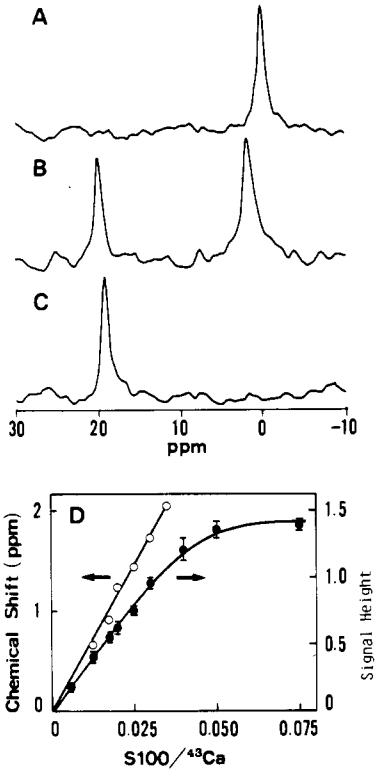

Figure 1. ^{43}Ca NMR spectra of 1.45 mM $^{43}Ca^{2+}$ (A), 1.45 mM $^{43}Ca^{2+}$–0.02 mM $S100_b$ protein (B), and 1.45 mM $^{43}Ca^{2+}$–0.11 mM $S100_b$ protein (C) in 0.15 M HEPES–K^+ buffer (pH 7.2). Signal heights were normalized to that of 1.45 mM $^{43}Ca^{2+}$. A total of 5×10^4 scans were acquired, and exponential line broadening of 20 Hz was applied. Part D shows changes in height of the signal at 19.2 ppm and of the chemical shift of the signal around 0.0 ppm caused by adding $S100_b$ protein. In part D, values along the x axis give the concentration ratio of [$S100_b$ protein]/[$^{43}Ca^{2+}$] and those along the y axis are signal height expressed in arbitrary units.

^{43}Ca NMR spectra of $^{43}Ca^{2+}$ (49.1%)–protein complexes were obtained as described previously[2] in 0.15 M *N*-(2-hydroxyethyl)piperazine-*N*'-ethanesulfonic acid (HEPES)–K^+ buffer (pH 7.2).

By addition of $S100_b$ protein to a 1.45 mM $^{43}Ca^{2+}$ solution, the ^{43}Ca NMR signal of the free $^{43}Ca^{2+}$ moved downfield by 2.0 ppm with concomitant increase of the line width (measured at half-height) from 0.5 Hz to more than 100 Hz (Figure 1A,B,D).[11] Simultaneously a new signal appeared at 19.2 ppm downfield from the signal of free $^{43}Ca^{2+}$ (Figure 1B). The increase of the peak height of the 19.2 ppm resonance saturated at the concentration ratio [$S100_b$]/[Ca^{2+}] = 0.050 (Figure 1D). For the $^{43}Ca^{2+}$ (1.45 mM)–$S100_b$ protein (0.11 mM) solution, only the signal at 19.2 ppm was observed (Figure 1C). Since Mg^{2+} is known to bind to S100 proteins,[3] excess Mg^{2+} was added to the Ca^{2+}–$S100_b$ solution to determine whether the Ca^{2+}- and Mg^{2+}-binding sites are the same. The signal at 19.2 ppm decreased by half, and a new signal appeared at 2.1 ppm upon addition of 50 mM Mg^{2+} to the Ca^{2+} (1.45 mM)–$S100_b$ (0.11 mM) solution.

When $S100_{a0}$ protein was added to the $^{43}Ca^{2+}$ (1.45 mM) solution, a new signal appeared at 19.3 ppm downfield from the free $^{43}Ca^{2+}$ signal (at 0.0 ppm) (Figure 2A,B). The appearance of the new signal at 19.3 ppm was concomitant with the shift of the resonance from 0.0 to 5.8 ppm and increased broadening of the original signal (now at 5.8 ppm).[11] The peak height of the

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