an important beginning for this research. A number of other novel applications also seem possible (*17*). For example, it appears feasible to collect a small number of atoms generated in rare nuclear decay events onto a surface. Present efforts are aimed toward determining the ββ-decay half-life of ^{136}Xe, a process predicted to yield annually one atom of $^{136}Ba^{2+}$ for every 8×10^{20} atoms of ^{136}Xe (*18*).

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Submicrometer Superconducting $YBa_2Cu_3O_{6+x}$ Particles Made by a Low-Temperature Synthetic Route

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Evidence suggests that superconducting, orthorhombic $YBa_2Cu_3O_{6+x}$ ($x \gtrsim 0.5$) is always produced by oxidation of the oxygen-deficient, tetragonal form ($x \lesssim 0.5$) of this phase (commonly referred to as 123). A synthetic route whereby solution-derived, carbon-free precursors are decomposed at 650° to 700°C in inert atmosphere to yield tetragonal 123 is now available. Appropriate precursors include hydrated oxides derived from the hydrolysis of organometallic solutions and aqueous solution-derived hyponitrites. Subsequent oxidation of the tetragonal phase at 400°C results in submicrometer particles of orthorhombic 123. Superconductivity (T_c onset ≈ 87 K) has been confirmed in these materials by both Meissner effect and specific-heat measurements.

THE PRACTICAL UTILIZATION OF $YBa_2Cu_3O_{6+x}$, will, in many cases, be dependent upon the ability to sinter powders of this material into strong dense compacts. Because sintering is driven by a decrease in surface free energy, good sinterability requires a powder comprised of small particles. The conventional solid-state synthesis of the $YBa_2Cu_3O_{6+x}$ (123) superconductor typically involves combining yttrium oxide, copper oxide, and barium carbonate, and heating, the mixture to about 950°C. This procedure produces a powder with large particles and poor sinterability (*1*, *2*).

Solution routes have been devised to prepare precursors that can be decomposed at relatively mild temperatures to yield finer particles of 123; however, decomposition temperatures of at least 800° to 850°C have been required to obtain single phase products (*2–8*). The stability of $BaCO_3$ represents a serious impediment to further lowering of the 123 synthesis temperature. Most of the reported synthetic routes to 123 employ $BaCO_3$ as one of the ingredients or other carbon-containing reagents, which, upon decomposition, convert to $BaCO_3$. Complete reaction of this $BaCO_3$ is never obtained at temperatures lower than 800°C, even with fine particle precursors. Trace amounts of unreacted $BaCO_3$ at the grain boundaries of a sintered compact are known to adversely affect critical current (*9*). The formation of $BaCO_3$ can be avoided all together if one uses precursors or reactants that do not contain carbon, and if the firing is performed in CO_2-free air or oxygen. However, there have been no well-documented successes in preparing essentially single phase, superconducting 123 powder by direct decomposition of carbon-free precursors at low temperatures (substantially below 800°C).

This lack of success in attempts at low-temperature synthesis, even in the absence of $BaCO_3$, is related to the preference of superconducting 123 to form by way of topotactic oxidation of the tetragonal phase. In all well-documented examples of 123 powder preparation, the synthesis has been carried out in air or oxygen at temperatures exceeding the orthorhombic to tetragonal phase transformation to yield $YBa_2Cu_3O_{6+x}$, where $x \approx 0.3$. It is only during cooling (or a subsequent low-temperature anneal) that the oxygen-deficient phase incorporates additional oxygen so as to make the material orthorhombic and superconducting ($x \gtrsim 0.5$). Explanations based on either kinetic (*10*) or thermodynamic (*11*) arguments may be offered to account for the difficulty in directly producing the orthorhombic phase. Whatever the correct explanation, it appears that low-temperature synthesis attempts should focus on the tetragonal, or low oxygen content phase.

We have found that it is possible to conduct low-temperature synthesis of 123 powder by first decomposing carbon-free precursors in inert gas to form the tetragonal $YBa_2Cu_3O_{6+x}$ (where $x \lesssim 0.5$). This phase can subsequently be annealed in oxygen to convert it to the superconducting, orthorhombic form ($x \gtrsim 0.5$). Manthiram and Goodenough (*12*) also recognized that a low-temperature, inert atmosphere anneal would yield a tetragonal phase suitable for subsequent oxidation to the superconducting orthorhombic form. In their case, however, the use of a carbon-containing precursor required that the argon anneal be preceded by an extended air calcine at 780°C in order to eliminate most of the $BaCO_3$. In the present study the use of carbon-free

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hyponitrite precursors or hydrated metal oxides derived from the hydrolysis of organometallic solutions allows the complete avoidance of $BaCO_3$ and thus, lower synthesis temperatures.

The hyponitrite precursors were precipitated from a nitrate solution of the desired stoichiometry (0.1*M* with respect to Y) by addition of a 0.3*M* aqueous $Na_2N_2O_2$ solution. Analytical results were consistent with the molecular formula $YBa_2Cu_3(N_2O_2)_{2.6}OH_xO_y$, where x and y were not determined. X-ray diffraction showed the precursor to be an unidentified, poorly crystalline solid.

The organometallic-derived precursors were prepared by controlled hydrolysis of homogeneous tetrahydrofuran (THF) solutions containing $Y(OCHMe_2)_3$, $Ba(OCHMe_2)_3$, and a hydrolyzable copper compound such as $Cu(OCMe_3)$, Cu(mesityl), $Cu(NBu_2)$, $Cu(OCH_2CH_2NEt_2)$, or $Cu(OCH_2CH_2OBu)_2$ (Me = methyl, Et = ethyl, and Bu = butyl). The later two compounds are unusual examples of soluble Cu(II) alkoxides, and were prepared by alcohol exchange with $Cu(OMe)_2$: $Cu(OCH_2CH_2NEt_2)_2$. $Cu(OMe)_2$ (2.00 g) was combined with 10 ml of *N,N*-diethylethanolamine and 40 ml of toluene, and heated to 100°C for 15 minutes to give a dark purple solution. The warm solution was filtered and the solvents were removed in vacuo to give a dark green solid. Sublimation of the crude product at 100°C in high vacuum gave 2.86 g of blue-green solid (75%) with an electron spin resonance spectrum typical of monomeric Cu(II). Elemental analysis [found (calculated)]: 41.63% C (43.69), 7.44% H (7.94), and 19.6% Cu (19.3). $Cu(OCH_2CH_2OBu)_2$ was prepared similarly by alcohol exchange of $Cu(OMe)_2$ with 2-butoxyethanol, and was purified by recrystallization from toluene. Elemental analysis: 47.9% C (48.39), 8.52% H (8.80), and 21.7% Cu (21.33).

A typical Cu(I)-containing metal-oxide precursor was prepared as follows: A solution of $Y(OCHMe_2)_3$ (5.09 g) $Ba(OCHMe_2)_2$ (9.77 g), and $Cu(NBu_2)$ 11.0 g) in 175 ml of THF was added dropwise to a solution of degassed water (17.2 g) in 175 ml of THF. The mixture was refluxed under argon for 16 hours, and filtered to give an orange solid. The solid was washed with THF followed by pentane, and dried at 100°C in vacuo to give 13.2 g of orange powder. Elemental analysis of the powder gave a molar ratio of Y:Ba:Cu = 1.00:2.02:3.00 with a carbon content of 0.2 to 0.3 wt.%. The carbon content was unchanged after a 10-minute exposure to ambient atmosphere but increased to 0.80% and 3.56% after 2 hours and 24 hours exposure to ambient, respectively. To prevent the formation of $BaCO_3$ during synthesis, the precursor was stored in inert atmosphere prior to use. Cu(II)-containing precursors were prepared by similar procedures with the soluble Cu(II) alkoxides.

Conversion of the hyponitrite and Cu(II)-containing hydrolyzed organometallic precursors was achieved by firing the precursors in flowing argon at 20°C per minute to 650° to 700°C for a period of 2 to 12 hours to yield tetragonal $YBa_2Cu_3O_{6+x}$ which was subsequently oxidized to the orthorhombic phase by annealing in flowing oxygen at 400°C for a period of 2 to 12 hours. The precursors were fired in thin layers (2 to 4 mm thick), and temperatures were measured with a thermocouple position ~1 cm directly above the sample. Since Cu(I)-containing precursors do not contain enough oxygen to support the $YBa_2Cu_3O_{6+x}$ stoichiometry, they were preoxidized at 300°C in oxygen for 2 hours before firing in argon.

Electron microscopy was carried out on the synthesized materials with both a Philips CM12 and a Hitachi H-600. Some samples were supported on carbon grids and examined without further preparation. Other samples were embedded in an epoxy and sectioned (60 to 80 nm thick) with an ultramicrotome with a 45° diamond knife. These sections were picked up on a 200-mesh copper grid with a carbon support film.

Meissner effect (flux expulsion) measurements were carried out by cooling the samples in a 20 G field from 120 to 7 K. A superconducting quantum interference device (SQUID) S.H.E. model 905 susceptometer was used for the measurement.

The specific heat was measured from 40 to 130 K in a semiadiabatic calorimeter. A more detailed discussion of the measurements will be given elsewhere (*13*).

All of the carbon-free precursors can be converted to tetragonal 123 at temperatures of 650° to 700°C. A sample prepared by hydrolysis of a mixture of $Y(OCHMe_2)_3$, $Ba(OCHMe_2)_2$, and $Cu(NBu_2)$ was chosen for detailed characterization. Figure 1a shows an x-ray diffraction pattern of tetragonal 123 obtained by oxidizing this hydrolyzed, Cu(I)-containing, organometallic precursor at 300°C, followed by decomposition at 700°C in argon. A trace amount of $BaCuO_2$ is also evident. A subsequent anneal at 400°C in oxygen results in conversion of the tetragonal 123 to the orthorhombic form, as shown in Fig. 1b. An x-ray diffraction pattern of orthorhombic 123 prepared by carrying out the argon decomposition at 650°C followed by a 400°C anneal in oxygen is shown in Fig. 1c. Fur-

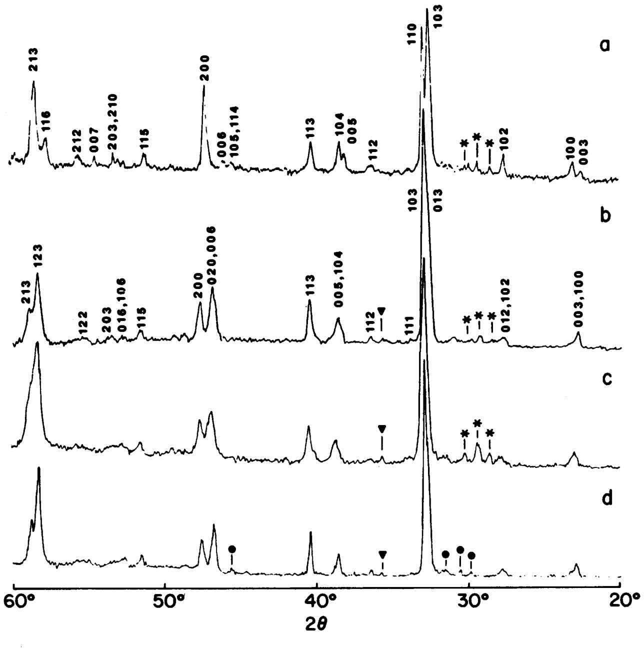

Fig. 1. X-ray diffraction patterns of 123 phases formed by conversion of the Cu(I)-containing precursor under the specified conditions: (**a**) 300°C in O_2 for 2 hours followed by 700°C in argon for 12 hours; (**b**) sample used for (a) annealed at 400°C in oxygen for 12 hours; (**c**) 300°C in oxygen for 2 hours, 650°C in argon for 12 hours, followed by an oxygen anneal at 400°C for 4 hours; and (**d**) sample used for (c) annealed at 950°C in oxygen for 4 hours. Second phases identified as follows: * = $BaCuO_2$, ▼ = CuO, ● = Y_2BaCuO_5.

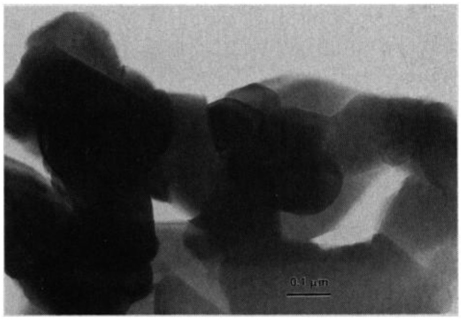

Fig. 2. Transmission electron microscope (TEM) image of orthorhombic 123 sample whose x-ray diffraction pattern is shown in Fig. 1b. Precursor decomposition temperature = 700°C.

ther annealing this material at 950°C in oxygen results in nearly phase-pure 123 as shown in Fig. 1d. When the precursor decomposition is conducted in oxygen, rather than argon, the 123 phase will not form below 700°C, and at that temperature the phase purity is quite poor.

Figure 2 is a transmission electron microscopy (TEM) image of the organometallic-derived, orthorhombic 123 whose x-ray diffraction pattern is shown in Fig. 1b. The powder is shown to consist of agglomerates of primary particles whose dimensions range between 100 and 500 nm with some occasionally larger crystallites. These primary particles are lightly sintered together resulting in extensive areas of grain boundary contact, but considerable porosity remains. In general the majority of the grains were in (110) and (120) crystal orientations, with a few occurring in (100) and (001) orientations. In agreement with the x-ray diffraction results, some unreacted crystals of copper oxide were also observed. Electron diffraction patterns were indistinguishable from similar patterns obtained on orthorhombic 123 made by conventional solid-state reaction (950°C). Remarkably, no significant twinning was observed. The majority of the 123 grains were defect-free, single crystals. The grains exhibited surface topography with faceting, surface steps and terraces on the surfaces of the grains as revealed by both SEM and TEM, indicating some degree of anisotropy in the surface energy term. The grain boundaries, which have previously been shown to strongly influence electrical transport properties (*14*), were found to be predominantly smooth and clean with no intervening amorphous layers or inpurities. A few grains with an enlarged *c* dimension of ~1.35 nm coexisting with the 123 phase were observed, suggesting the presence of a phase similar to $YBa_2Cu_4O_8$ (*15*). The occurrence of these grains, however, was not common.

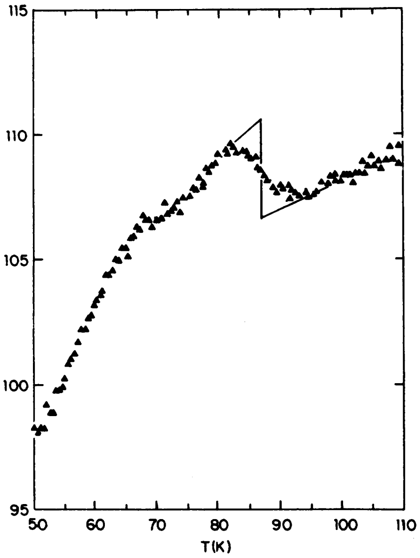

Fig. 3. Specific heat versus temperature for orthorhombic 123 phase characterized in Fig. 1b and Fig. 2. Specific-heat jump, denoted by dotted line, is determined according to discussion given in text.

The specific-heat data for the above sample, shown in Fig. 3, exhibits a broad anomaly below ~ 90 K. Both the location and magnitude of the discontinuity are determined by extrapolation from above and below the anomaly and are defined at the point for which the entropy near the transition is balanced. The discontinuity in the specific heat is found to be 290 mJ/(g-at. K) at 87 K, which is approximately 70 to 90% of the values reported for single-phase superconducting $YBa_2Cu_3O_{6+x}$ samples in the literature (*16, 17*). This measurement was repeated in the transition region of 75 to 95 K, and a specific-heat jump of identical magnitude was reproduced. The magnetic susceptibility data, shown in Fig. 4, indicates a transition temperature (T_c) onset of ~85 K, consistent with the heat capacity results. In contrast to the specific-heat experiment that measures bulk superconductivity in the absence of magnetic field, the small diamagnetic signal observed (~1.3% of ideal Meissner signal) can be attributed to the small particle size to magnetic-field penetration depth ratio (*13*).

Decreasing the temperature of the inert-atmosphere decomposition of the precursor to 650°C permits the synthesis of still finer particles of orthorhombic 123 (x-ray diffraction pattern in Fig. 1c) as shown in the TEM image of Fig. 5. These particles range in size from 40 to 200 nm and are much less aggregated than those prepared with the slightly higher precursor decomposition temperature. In contrast to the material shown in Fig. 2, the particles seen here are

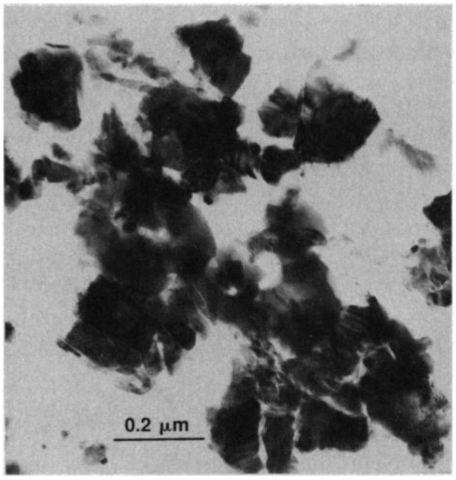

Fig. 5. TEM image of orthorhombic 123 sample whose x-ray diffraction pattern is shown in Fig. 1c. Precursor decomposition temperature = 650°C.

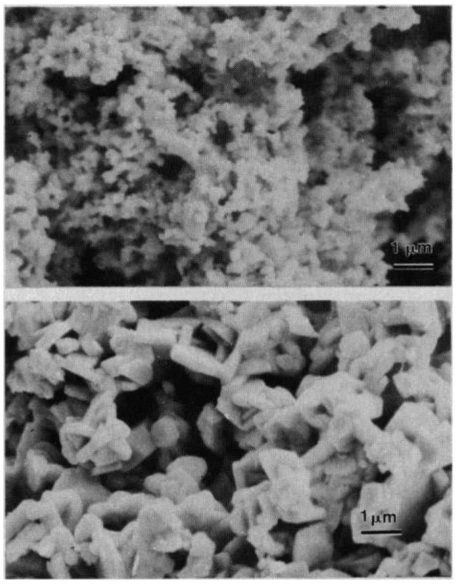

Fig. 6. Scanning electron microscope (SEM) image of (**top**) as-synthesized 123 sample shown in Fig. 5 and (**bottom**) after annealing in oxygen at 950°C for 4 hours.

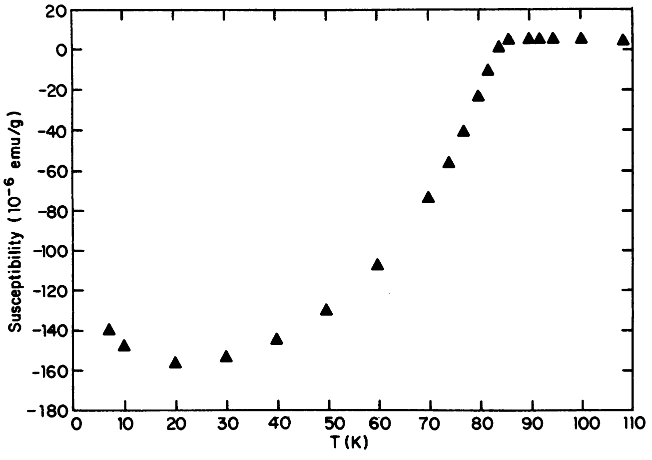

Fig. 4. Magnetic susceptibility versus temperature for orthorhombic 123 characterized in Figs. 1b and 2.

twinned along (110) boundaries as is typical with this phase. The reason for the formation of defect-free 123 under certain conditions of precursor conversion remains unresolved and is presently under investigation. The observation of superconductivity in defect-free crystals demonstrates, however, that twinning is not a requirement of superconductivity in the $YBa_2Cu_3O_{6+x}$ system. When the powder shown in Fig. 5 and Fig. 6 (top) is further annealed at 950°C in 4 hours in oxygen, the particles, as seen in Fig. 6 (bottom), remain quite small, ranging in size from 500 to 2000 nm. Moreover, there is no evidence of any liquid phase formation. These observations suggest potential advantages with respect to sintered microstructure.

While we are not aware of any previous, documented examples of superconducting 123 powder prepared at temperatures substantially less than 800°C, there have been several reports that superconducting 123 can be made at low temperatures (for example, 600°C) by thin-film techniques (*18, 19*). However, in a typical thin-film synthesis, the temperature quoted is the substrate temperature, which can be considerably lower than the actual temperature at the surface of the growing film. Moreover, the substrate temperatures reported are about equal to or in excess of the orthorhombic to tetragonal phase transformation temperature in the range of pO_2 (0.003 to 0.35 atm) employed (*20*). Thus, a thin-film synthesis may be regarded as a two-step process, first yielding what is most likely nonsuperconducting tetragonal 123, with oxidation to the orthorhombic form occurring during cooling.

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Effects of Buried Ionizable Amino Acids on the Reduction Potential of Recombinant Myoglobin

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The temperature dependences of the reduction potentials ($E^{\circ\prime}$) of wild-type human myoglobin (Mb) and three site-directed mutants have been measured by the use of thin-layer spectroelectrochemistry. Residue Val^{68}, which is in van der Waals contact with the heme in Mb, has been replaced by Glu, Asp, and Asn. The changes in $E^{\circ\prime}$ and the standard entropy ($\Delta S^{\circ\prime}$) and enthalpy ($\Delta H^{\circ\prime}$) of reduction in the mutant proteins were determined relative to values for wild type; the change in $E^{\circ\prime}$ at 25°C was about −200 millivolts for the Glu and Asp mutants, and about −80 millivolts for the Asn mutant. At *p*H 7.0, reduction of Fe(III) to Fe(II) in the Glu and Asp mutants is accompanied by uptake of a proton by the protein. These studies demonstrate that Mb can tolerate substitution of a buried hydrophobic group by potentially charged and polar residues and that such amino acid replacements can lead to subsantial changes in the redox thermodynamics of the protein.

WE REPORT MEASUREMENTS OF the temperature dependences of the reduction potentials of wild-type and three single-site mutants of human myoglobin (Mb). Specifically, we have investigated mutations in which Val^{68} has been changed to the potentially charged residues Glu and Asp, and to Asn, which is uncharged and polar, in order to evaluate the influences of the local electrostatic field on the redox properties of this protein.

These studies were motivated by extensive theoretical (*1, 2*) and experimental (*3, 4*) investigations of the degree to which a protein can influence the redox potential of a prosthetic group or stabilize separated charge after electron transfer. Important factors that affect the reduction potential of a protein include (i) the nature of ligands at the redox center (*3*), (ii) conformational changes associated with reduction, and (iii) electrostatic interactions of the redox center with charged groups both on the surface (*5*) and in the interior of the protein (*6*). Electrostatic interactions are affected by water molecules and ions both in solution and bound at specific sites on the protein and by dipolar and polarizable groups that are present within the protein (*7*). In a recent report, the magnitude of electrostatic interactions between pairs of charged residues was estimated in mutants of subtilisin (*8, 9*). These studies focused on interactions between residues that are accessible to aqueous solvent. However, little is known about the magnitudes of electrostatic interactions between charged groups that are buried within the protein. Since surface charges are well solvated by water, their interactions with the redox center are expected to be smaller than those of buried charges.

In order to assess the magnitude of various contributions to $E^{\circ\prime}$, studies have typically been made of structurally similar proteins and most extensively for the cytochromes (*4*). Such analyses are complicated in that the proteins studied differ at several positions in their primary amino acid sequence. With the advent of site-specific mutagenesis, the effects of individual amino acid changes on redox thermodynamics can be systematically studied. We have chosen Mb because it has been extensively characterized and because of the recent availability of a cDNA clone for human Mb and an efficient method for overproduction in *Escherichia coli* (*10*). Although an x-ray structure of human Mb is not yet available, the residues in the heme pocket are the same as those found in sperm whale Mb, and the optical, ligand binding, nuclear magnetic resonance (NMR), and redox properties of the two proteins are similar (*10*). Residue

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