Morphology-Dependent Electrochemistry of Cytochrome *c* at Au Colloid-Modified SnO_2 Electrodes

Kenneth R. Brown, Audrey P. Fox, and Michael J. Natan*

Contribution from the Department of Chemistry, The Pennsylvania State University, 152 Davey Laboratory, University Park, Pennsylvania 16802-6300

Received August 28, 1995[⊗]

Abstract: Reversible electrochemistry of horse heart cytochrome *c* (Cc) has been obtained at SnO_2 electrodes modified with 12-nm-diameter colloidal Au particles. Previous experiments had demonstrated electrochemical addressability of these particle ensembles; the high current densities and small peak-to-peak separations observed for Cc are indicative of facile electron transfer. In contrast to previously described modified electrodes for Cc, these data were acquired without polishing and without any modification of the Au particle surface. Quasireversible voltammetry was obtained with surfaces comprising monodisperse 36-nm-diameter and polydisperse 6-nm-diameter Au particles, but no voltammetric wave for Cc was seen at surfaces composed of aggregates of 12-nm-diameter or 22-nm-diameter Au particles. These data indicate that nanometer-scale morphology of metals plays a key role in protein electrochemistry, and suggest that isolated, surface-confined colloidal Au particles may be useful building blocks for macroscopic metal surfaces for biological applications.

Introduction

Direct electron transfer (ET) between an electrode and a redox protein, an important step for electrochemical biosensors,[1] requires interfaces that exhibit reasonably fast ET kinetics and are biocompatible (i.e. do not denature proteins). Since direct contact between redox proteins and uncoated metal surfaces usually leads to significant protein structural and/or functional changes,[2] the desired interfacial properties are accessible only through electrode[3] or protein[4] modification. Numerous applications of chemically-modified macroscopic electrodes to voltammetric measurements on horse heart cytochrome *c* (Cc)[5,6] and other redox proteins[7] have been described. A common theme in this work has been to prevent protein−metal surface contact. In contrast, direct contact between uncoated, nanometer-sized colloidal Au particles and proteins is common in histochemistry,[8] where it has been demonstrated that electrostatically-bound colloidal Au:protein conjugates typically retain biological activity.[9] Indeed, Crumbliss and co-workers have exploited this property in an electrochemical glucose biosensor.[10]

We show herein that direct, reversible cyclic voltammetry of horse heart cytochrome *c* (Cc) in solution is obtained at uncoated submonolayers of 12-nm-diameter colloidal Au particles[11] on SnO_2, without the need for any pretreatment or polishing steps. The colloidal particles behave as an ensemble of closely-spaced but isolated microelectrodes. However, when the size of electrode features increases through particle aggregation, Cc electrochemistry becomes quasireversible or irreversible. These results show the importance of nanometer-scale morphology in protein voltammetry, and demonstrate that well-

* Author to whom all correspondence should be addressed: E-Mail natan@chem.psu.edu.

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1996.

(1) (a) Scheller, F.; Schubert, F. *Biosensors*; Elsevier: New York, 1992. (b) Lambrechts, M.; Sansen, W. *Biosensors: Microelectrochemical Devices*; Institute of Physics Publishing: Philadelphia, 1992. (c) Albery, W. J.; Craston, D. H. In *Biosensors: Fundamentals and Applications*; Turner, A. P. F, Karube, I., Wilson, G. S., Eds.; Oxford University Press: London, 1987; pp 180−210. (d) Guo, L.-H.; Hill, H. A. O. In *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic Press: San Diego, CA, 1991; Vol. 36, p 341. (e) Armstrong, F. A. In *Advances in Inorganic Chemistry*; Sykes, A. G., Ed.; Academic Press: San Diego, CA, 1992; Vol. 38, p 117.

(2) (a) Holt, R. E.; Cotton, T. M. *J. Am. Chem. Soc.* **1989**, *111*, 2815−2821. (b) Yang, M.; Chung, F. L.; Thompson, M. *Anal. Chem.* **1993**, *65*, 3713−3716.

(3) (a) Armstrong, F. A. *Struct. Bonding (Berlin)* **1990**, *72*, 137−221. (b) Armstrong, F. A.; Hill, H. A. O.; Walton, N. J. *Acc. Chem. Res.* **1988**, *21*, 407−413. (c) Bard, A. J. *Pure Appl. Chem.* **1992**, *64*, 185−192. (d) Frew, J. E.; Hill, H. A. O. *Eur. J. Biochem.* **1988**, *172*, 261−269. (e) Harmer, M. A.; Hill, H. A. O. *J. Electroanal. Chem.* **1985**, *189*, 229−246.

(4) Heller, A. *Acc. Chem. Res.* **1990**, *23*, 128−134.

(5) (a) Yeh, P.; Kuwana, T. *Chem. Lett.* **1977**, 1145−1148. (b) Bowden, E. F.; Hawkridge, F. M.; Chlebowski, J. F.; Bancroft, E. E.; Thorpe, C.; Blount, H. N. *J. Am. Chem. Soc.* **1982**, *104*, 7641−7644. (c) Bowden, E. F.; Hawkridge, F. M.; Blount, H. N. *J. Electroanal. Chem.* **1984**, *161*, 355−376. (d) Reed, D. E.; Hawkridge, F. M. *Anal. Chem.* **1987**, *59*, 2334−2339. (e) Szücs, A.; Novák, M. *J. Electroanal. Chem.* **1995**, *383*, 75−84.

(6) (a) Allen, P. M.; Hill, H. A. O.; Walton, N. J. *J. Electroanal. Chem.* **1984**, *178*, 69−86. (b) Armstrong, F. A.; Bond, A. M.; Hill, H. A. O.; Oliver, B. N.; Psalti, I. S. M. *J. Am. Chem. Soc.* **1989**, *111*, 9185−9189. (c) Bartlett, P. N.; Farington, J. *J. Electroanal. Chem.* **1989**, *261*, 471−475. (d) Collinson, M.; Bowden, E. F.; Tarlov, M. J. *Langmuir* **1992**, *8*, 1247−1250. (e) Cooper, J. M.; Greenough, K. R.; McNeil, C. J. *J. Electroanal. Chem.* **1993**, *347*, 267−275. (f) Eddows, M. J.; Hill, H. A. O. *J. Am. Chem. Soc.* **1979**, *101*, 4461−4464. (g) Erabi, T.; Ozawa, S.; Hayase, S.; Wada, M. *Chem. Lett.* **1992**, 2115−2118. (h) Gleria, K. D.; Hill, H. A. O.; Lowe, J. V.; Page, D. J. *J. Electroanal. Chem.* **1986**, *213*, 333−338. (i) Haladjian, J.; Bianco, P.; Pilard, R. *Electrochim. Acta* **1983**, *28*, 1823−1828. (j) Hill, H. A. O.; Lawrence, G. A. *J. Electroanal. Chem.* **1989**, *270*, 309−318. (k) Hinnen, C.; Parsons, R.; Niki, K. *J. Electroanal. Chem.* **1983**, *147*, 329−337. (l) Lewis, N. S.; Wrighton, M. S. *Science* **1981**, *211*, 944−946. (m) Lu, T.; Yu, X; Dong, S.; Zhou, C.; Ye, S.; Cotton, T. M. *J. Electroanal. Chem.* **1994**, *369*, 79−86. (n) Oliver, B. N.; Egekeze, J. O.; Murray, R. W.; *J. Am. Chem. Soc.*, **1988**, *110*, 2321−2322. (o) Taniguchi, I.; Iseki, M.; Toyosawa, K.; Yamaguchi, H.; Yasukouchi, K. *J. Electroanal. Chem.* **1984**, *164*, 385−391. (p) Taniguchi, I.; Iseki, M.; Yamaguchi, H.; Yasukouchi, K. *J. Electroanal. Chem.* **1984**, *175*, 341−348. (q) Taniguchi, I.; Higo, N.; Umetika, K.; Yasukouchi, K. *J. Electroanal. Chem.* **1986**, *206*, 341−348.

(7) (a) Armstrong, F. A.; George, S. J.; Cammack, R.; Hatchikian, E. C.; Thomson, A. J. *Biochem. J.* **1989**, *264*, 265−273. (b) Armstrong, F. A.; Bond, A. M.; Hill, H. A. O.; Oliver, B. N.; Psalti, I. S. M. *J. Am. Chem. Soc.* **1989**, *111*, 9185−9189. (c) Armstrong, F. A.; Bond, A. M.; Büchi, F. N.; Hamnett, A.; Hill, H. A. O.; Lannon, A. M.; Lettington, O. C.; Zoski, C. G. *Analyst* **1993**, *118*, 973−978. (d) Haladijian, J.; Brushi, M.; Nunzi, F.; Bianco, P. *J. Electroanal. Chem.* **1993**, *253*, 329−335. (e) Harmer, M. A.; Hill, H. A. O. *J. Electroanal. Chem.* **1984**, 170, 269−375. (f) Schlereth, D. D.; Mäntele, W. *Biochemistry* **1992**, *31*, 7494−7502. (g) Salamon, Z.; Tollin, G. *Arch. Biochem. Biophys.* **1992**, *294*, 382−387. (h) Taniguchi, I.; Hirakawa, Y.; Iwakiri, K.; Tominaga, M.; Nishiyama, K. *J. Chem. Soc., Chem. Commun.* **1994**, 953−954. (i) Tominaga, M.; Kumagai, T.; Takita, S.; Taniguchi, I. *Chem. Lett.* **1993**, 1771−1774. (j) Zhao, J.; Henkens, R. W.; Stonehuerner, J.; O'Daly, J. P.; Crumbliss, A. L. *J. Electroanal. Chem.* **1992**, *327*, 109−119.

(8) Hayat, M. A., Ed. *Colloidal Gold: Principles, Methods, and Applications*; Academic Press: San Diego, 1989; Vols. 1−2.

0002-7863/96/1518-1154$12.00/0

defined, Au colloid-based substrates hold promise as substrates for biological measurements at metal surfaces.

Experimental Section

Reagents and Materials. All reagents were used as received except where noted. (3-Aminopropyl)trimethoxysilane (APTMS) was obtained from Hüls America. HCl, HNO_3, H_2SO_4, NaOH, NaH_2PO_4, Na_2HPO_4, KH_2PO_4, K_2HPO_4, and H_2O_2 were obtained from Baker. $K_3[Fe(CN)_6]$, $HAuCl_4 \cdot 3H_2O$, trisodium citrate dihydrate, $NaBH_4$, and Na_2SO_4 were purchased from Aldrich. $[Ru(NH_3)_6]Cl_3$ was purchased from Alfa. Absolute C_2H_5OH was obtained from Aaper Alcohol and Chemical Company. Spectrophotometric grade CH_3OH was obtained from EM Science. CM cellulose and horse heart Cytochrome *c* (Cc) were obtained from Sigma; Cc was purified according to the method of Brautigan et al.[12] Substrates were obtained as follows: In-doped SnO_2 (30 Ω-cm) from PPG Industries, Sb-doped SnO_2 (100 Ω-cm) from Delta Technology. All H_2O was 18 MΩ purified by a Barnstead Nanopure water purification apparatus.

Preparation of Colloidal Particles. All glassware used in the following procedures was cleaned in a bath of freshly prepared 3:1 HNO_3−HCl, rinsed thoroughly in H_2O, and dried prior to use. Preparations were stored in amber glass or polyethylene bottles.

A solution of polydisperse 6-nm-diameter Au colloidal particles was prepared by mixing 2.0 mL of 1% trisodium citrate and 0.75 mL of 0.075% $NaBH_4$/1% trisodium citrate with 500 mL of H_2O/5.0 mL of 1% $HAuCl_4$ solution at room temperature. Solutions of 12-, 22-, and 36-nm-diameter colloidal Au particles were prepared as previously described.[11] Particle-size statistics: (**A** and **D**) 12 nm ± 1.6 nm, 284 particles counted; (**B**) 36 nm ± 5 nm (major axis), 29 nm ± 3 nm (minor axis), 436 particles counted; (**C**) 6 nm ± 4 nm, 1450 particles counted, largest particle diameter = 21 nm, smallest particle diameter = 2 nm; (**E**) 23 nm ± 4 nm, 47 particles counted. Methods for determination of particle size are described elsewhere.[11]

Preparation of SnO_2 Electrodes. Cu wires were affixed to the conducting surfaces of In- or Sb-doped SnO_2 using Ag epoxy. The Cu wires were encased in glass, and any exposed Ag epoxy or Cu was insulated with Dexter Epoxi-Patch. Electrodes were cleaned by sequential sonications in acetone, soap, and distilled H_2O (2×, 15 min each) and were stored in H_2O after electrochemical characterization (cyclic voltammetry of 5 mM $K_3[Fe(CN)_6]$ in 0.1 M aqueous Na_2SO_4). Au colloid monolayers were prepared on these electrodes as follows: After exposure to piranha solution for 5 min, electrodes were sonicated for 20 min each in H_2O and acetone, followed by 15 min of sonication in H_2O. The electrodes were then treated with 5 M NaOH for 5 h prior to rinsing with H_2O and derivatization in an aqueous solution of 1% APTMS for 10−15 min. Substrates were rinsed in H_2O and allowed to dry in air. Subsequent exposure to colloidal Au resulted in colloid binding; further details may be found elsewhere.[11,17]

Optical Instrumentation. Optical spectra were recorded using an HP8452A diode array ultraviolet−visible spectrophotometer with 2-nm resolution and a 1-s integration time. A Teflon block (1/3 sample height) was used to keep samples upright in cuvettes.

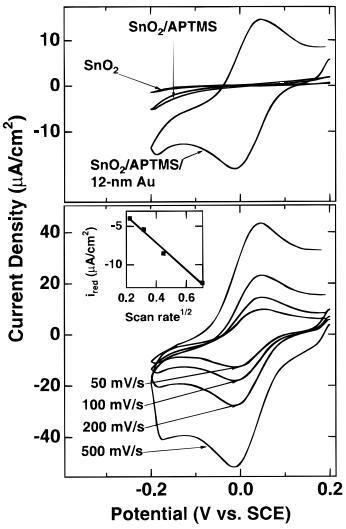

Figure 1. Top: Cyclic voltammograms at 100 mV/s of 0.5 mM Cc in 0.1 M $NaClO_4$/18 mM, pH 7 Na_2HPO_4/NaH_2PO_4 at SnO_2, at APTMS-coated SnO_2, and at APTMS-coated SnO_2 derivatized with 12-nm-diameter colloidal Au. Bottom: Scan rate dependence for 12-nm-diameter colloidal Au-modified electrode. Inset: Graph of cathodic peak current (i_{red}) vs (scan rate)$^{1/2}$.

Electrochemical Instrumentation. All electrochemical measurements were carried out using either a Cypress Model CS-1090 potentiostat operated with Version 6.0 Software on a Swan Technologies 386SX IBM-compatible computer or a PAR Model 273A Potentiostat/Galvanostat operated with Model 270 Software on a Gateway 486 IBM-compatible computer.

Results and Discussion

Facile ET between Cc in solution and an Au colloid submonolayer on SnO_2 is shown by the cyclic voltammetry (CV) data in Figure 1. At untreated or APTMS-coated SnO_2 electrodes, no voltammogram is observed from a solution containing 0.5 mM Cc in supporting electrolyte. Since similarly-prepared electrodes yield reversible voltammetry for $[Ru(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{3-}$, the absence of a redox wave for Cc derives from an overpotential specific to this particular couple at these interfaces, as opposed to poor electrode kinetics in general. Such behavior accords with the majority of previous voltammetric measurements on Cc, in which no ET is seen without special pretreatment.[5,6,13]

However, when roughly 15% of a monolayer of 12-nm-diameter colloidal Au particles are covalently attached to the amine groups of the SnO_2-confined polymer derived from APTMS,[11] reversible voltammetry of Cc is obtained. Note that the presence of a depletion region in the voltammogram is expected for an ensemble of closely-spaced microelectrodes.[14] Peak currents are proportional to the square root of scan rate (Figure 1, lower panel); for the best electrodes, the roughly 60-mV peak-to-peak separation (ΔE_p) is consistent with theoretical

(9) (a) Geoghegan, W. D.; Ackerman, G. A. *J. Histochem. Cytochem.* **1977**, *25*, 1187−1200. (b) Bendayan, M. In *Colloidal Gold: Principles, Methods, and Applications*; Hayat, M. A., Ed.; Academic Press: San Diego, 1989; Vol. 2, Chapter 2.

(10) (a) Crumbliss, A. L.; Perine, S. C.; Stonehuerner, J.; Tubergen, K. R.; Zhao, J.; Henkens, R. W.; O'Daly, J. P. *Biotechnol. Bioeng.* **1992**, *40*, 483−490. (b) Crumbliss, A. L.; Stonehuerner, J. G.; Henkens, R. W.; Zhao, J.; O'Daly, J. P. *Biosens. Bioelectron.* **1993**, *8*, 331−337. (c) Zhao, J.; Henkens, R. W.; Stonehuerner, J.; O'Daly, J. P.; Crumbliss, A. L. *J. Electroanal. Chem.* **1992**, *327*, 109−119.

(11) (a) Freeman, R. G.; Grabar, K. C.; Allison, K. J.; Bright, R. M.; Davis, J. A.; Guthrie, A. P.; Hommer, M. B.; Jackson, M. A.; Smith, P. C.; Walter, D. G.; Natan, M. J. *Science* **1995**, *267*, 1629−1632. (b) Grabar, K. C.; Freeman, R. G.; Hommer, M. B.; Natan, M. J. *Anal. Chem.* **1995**, *67*, 735−743. (c) Grabar, K. C.; Deutsch, J. E.; Natan, M. J. *Polym. Prepr.* **1995**, 69−70. (d) Bright, R. M.; Walter, D. G.; Musick, M. D.; Jackson, M. A.; Allison, K. J.; Natan, M. J. *Langmuir* In press. (e) Grabar, K. C.; Allison, K. J.; Baker, B. E.; Bright, R. M.; Brown, K. R.; Dolan, C. M.; Freeman, R. G.; Fox, A. F.; Musick, M. D.; Natan, M. J., *Langmuir* Accepted for publication.

(12) Brautigan, D. L.; Ferguson-Miller, S.; Margoliash, E. *Methods. Enzymol.* **1978**, *53*, 128−164.

(13) Direct electrochemistry of Cc has been observed at Ag, In_2O_3, and SnO_2 electrodes.[5] For the latter two, such measurements are dependent on experimental conditions, electrode pretreatment and history, and variability in commercial batches of metal oxides. It may be possible to find conditions under which the Sb- and In-doped SnO_2 used here give reversible electrochemistry of Cc, but we have not yet elucidated them.

(14) Amatore, C.; Savéant, J. M.; Tessier, D. *J. Electroanal. Chem.* **1983**, *147*, 39−51. At conventional scan rates, microelectrode behavior onsets when the fraction of surface covered with microelectrodes is ≈0.001. At the very high coverages of colloid used here, scan rates far in excess of 10^6 V/s would be needed to see steady-state voltammograms from radial diffusion.

Table 1. Peak Potentials ($E_{p,red}$; $E_{p,ox}$),Peak-to-Peak Separations (ΔE_p), and Redox Potentials ($E^{o\prime}$) for Cc on 12-nm Diameter Au Colloid Monolayer Electrodes

electrode no.	$E_{p,red}$ (mV)[a]	$E_{p,ox}$ (mV)[a]	ΔE_p (mV)[a]	$E^{o\prime}$ (mV)[a,b]
1	−12	48	60	18
2	1	62	61	31
3	7	70	63	39
4	1	72	71	37
5	−11	66	77	27
6	−21	73	94	26
7	−25	71	96	23
8	−30	71	101	21
9	−30	71	101	21
10	−16	85	101	35
11	−25	78	103	27
12	−23	87	110	32
13	−30	82	112	26
14	−29	86	115	28

[a] Versus SCE. [b] The average $E^{o\prime}$ is 27 ± 6 mV.

expectations.[15] From the slight increase in ΔE_p with scan rate, a heterogeneous rate constant of 7×10^{-3} cm s^{-1} is calculated,[15b] a value similar to others reported.[5,6] It should be noted that the peak current densities for reduction (15−20 μA/cm^2) are nearly identical to those obtained for $[Ru(NH_3)_6]^{3+}$ at the same electrode, when differences in solution concentration and diffusion coefficient are taken into account. The ability to directly record reasonably large currents for redox proteins at electrodes that require no polishing, coating, or other pretreatment is a significant advance.

Previous work on electrochemistry of redox metalloproteins at metal electrodes has identified two routes by which ET can be facilitated: via "mediators" (discrete, electroactive intermediaries between electrodes and solution couples) or "promoters" (electroinactive facilitators of direct ET between electrodes and solution couples).[3] Since colloidal Au is faradaically inactive, and since it has been previously demonstrated that electron transfer occurs directly at the Au/solution interface in Au colloid submonolayers,[11] colloidal Au fits neither of these descriptions. Rather, these small particles could be thought of as "electron antennae", efficiently funneling electrons between the electrode and the electrolyte. Moreover, the intrinsic negative charge on colloidal Au and the presence of a positive dipole moment around the heme cleft of Cc[16] may facilitate close approach of the protein to the colloid surface.

The reproducibility and persistence of the Cc voltammetric signal are addressed by the data in Table 1 and Figure 2. Table 1 lists peak potentials, ΔE_p, and $E^{o\prime}$ for 14 different electrodes modified with 12-nm Au particles. Note that 100% of the electrodes gave a voltammetric signal for Cc, with ΔE_p values varying from 60 to 115 mV. Figure 2 shows representative voltammograms for electrodes with high and low ΔE_p values. Voltammetric waves that are initially broad (e.g. >100 mV) usually yielded successively poorer voltammograms, while those that are initially narrow (e.g. <100 mV) typically exhibited a scan-number invariant signal.

The known sensitivity of protein stability to Au particle size and aggregation state[8] suggested investigation of four additional electrode surface modifications: 36-nm-diameter (**B**) and 6-nm (**C**) *isolated* colloidal Au particles, along with a pair composed of *aggregated* 12-nm (**D**) and 22-nm (**E**) colloidal Au. The

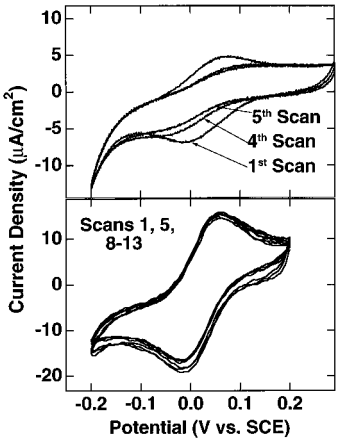

Figure 2. Reproducibility of the Cc voltammetric signal (100 mV/s) at two 12-nm colloidal Au-modified SnO_2 electrodes. See Figure 1 for conditions.

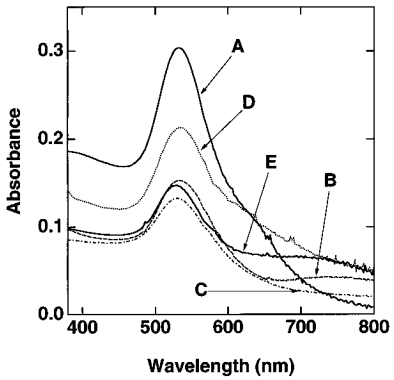

Figure 3. Optical spectra for SnO_2 electrodes derivatized with (**A**) isolated 12-nm-diameter, (**B**) isolated 36-nm-diameter, (**C**) isolated polydisperse 6-nm-diameter, (**D**) aggregated 12-nm-diameter, and (**E**) aggregated 22-nm-diameter colloidal Au particles.

degree of aggregation of colloidal Au particles in this size regime can be ascertained by examination of optical properties between 700 and 800 nm (Figure 3).[17] The presence of a low-energy tail or distinct peak (as in **D**, **E**, and to a lesser extent **B**) corresponds to closely-spaced or flocculated particles; increasing ratios of this low-energy feature to the 520-nm band signifies increasing interaction between particles (i.e. decreased spacing).

Figure 4 reveals a surprising dependence of the voltammetric behavior of Cc on nanometer-scale morphology. The letters **A**−**E** on the five voltammograms shown correspond to optical characterization data in Figure 3. Surfaces derivatized with isolated 36- and 6-nm-diameter Au colloidal particles give rise to reasonably good voltammetry, but with larger average peak-to-peak separations than for electrodes derivatized with 12-nm-diameter particles. In contrast, surfaces composed of aggregated 12-nm-diameter Au particles yield a barely discernible redox wave for Cc, and surfaces with more extensive particle aggregation exhibit rectifying behavior in Cc solutions (not shown). Similarly, electrodes with aggregates of 22-nm Au particles give no electrochemical signal for Cc. These dramatic differences cannot be accounted for solely by differences in surface area: it has been shown elsewhere that Beer's law holds for 12-nm Au particles in this coverage regime,[18] meaning that samples **A** and **D**, for example, differ in overall coverage by

(15) (a) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods;* John Wiley & Sons: New York, 1980. (b) Rieger, P. H. *Electrochemistry;* Prentice-Hall; Englewood Cliffs, NJ, 1987.

(16) (a) Swanson, R.; Trus, B. L.; Mandel, N.; Mandel, G.; Kallai, O. B.; Dickerson, R. E. *J. Biol. Chem.* **1977**, *252*, 759−775. (b) Takano, T.; Trus, B. L.; Mandel, N.; Mandel, G.; Kallai, O. B.; Swanson, R.; Dickerson, R. E. *J. Biol. Chem.* **1977**, *252*, 776−785.

(17) Blatchford, C. G.; Campbell, J. R.; Creighton, J. A. *Surf. Sci.* **1982**, *120*, 435−455.

(18) (a) Grabar, K. C.; Smith, P. C.; Davis, J. A.; Musick, M. D.; Jackson, M. A.; Walter, D. G.; Guthrie, A. P.; Natan, M. J. **1996**, *118*, 1148−1153. (b) Grabar, K. C.; Natan, M. J. Manuscript in preparation.

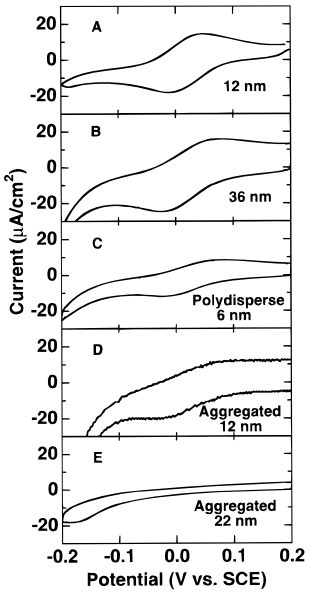

Figure 4. Cc voltammetry (100 mV/s) at the five modified SnO_2 electrodes described in Figure 3. See Figure 1 for conditions.

only ≈50%. It would thus appear that the Au nanoparticle aggregation state is a key determinant of electrochemical behavior toward Cc. Previous studies have cited irreversible Cc adsorption as the cause of poor electrochemistry;[3,5,6] by analogy, particle aggregates could provide sites for irreversible protein adsorption. These findings accord with previously described histochemical data on colloidal Au.[8]

Clearly, direct correlation of electrochemical behavior with nanometer-scale structure would be invaluable. Colloidal Au particles on SnO_2 electrodes have previously been observed by tapping-mode atomic force microscopy,[11d] but intrinsic substrate roughness precluded meaningful analysis of interparticle spacing: images of surfaces indicating extensive aggregation by UV−vis were not significantly different from those containing isolated particles. However, recent high-resolution field emission scanning electron microscopy studies of colloidal Au particles on transparent, rough silane films[18] shows promise as a technique for determining the nanostructure of Au assemblies on SnO_2.

Conclusions

Reversible voltammetry of Cc in solution has been obtained at SnO_2 electrodes modified with isolated colloidal Au particles, but surfaces with aggregates of particles are ineffective for the same task. The favorable ET properties of arrays of isolated nanometer-scale Au particles indicate that they may be generally useful for protein voltammetry. In addition, with the detailed understanding of Au colloid submonolayers now available,[11,18] a colloidal building-block approach to Au surfaces for other biological applications such as surface plasmon resonance,[19] quartz crystal microgravimetry,[20] and surface enhanced Raman scattering[21] is now feasible. Work along these lines is now in progress.

Acknowledgment is made to the National Science Foundation (CHE 92-08614, CHE 92-56692, and CHE 93-07485) and the Beckman Foundation for partial support of this research. Acknowledgment is also made to the Electron Microscopy Facility for the Life Sciences in the Biotechnology Institute at The Pennsylvania State University.

JA952951W

(19) (a) Löfås, S.; Johnsson, B.; Tegendal, K.; Rönnberg, I. *Colloids Surf. B: Biointerfaces* **1993**, *1*, 83−89. (b) Löfås, S. *Pure Appl. Chem.* **1995**, *67*, 829−834. (c) Ward, L. D.; Howlett, G. J.; Hammacher, A.; Weinstock, J.; Yasukawa, K.; Simpson, R. J.; Winzor, D. J. *Biochemistry* **1995**, *34*, 2901−2907. (d) Plant, A. L.; Brigham-Burke, M.; Petrella, E. C.; O'Shannessy, D. J. *Anal. Biochem.* **1995**, *226*, 342−348.

(20) (a) Buttry, D. A.; Ward, M. D. *Chem. Rev.* **1992**, *92*, 1355−1379. (b) Lacour, F.; Torresi, R.; Gabrielli, C.; Caprani, A. *Colloids Surf. B: Biointerfaces* **1993**, *1*, 251−259. (c) Minunni, M.; Skládal, P.; Mascini, M. *Anal. Lett.* **1994**, *27* 1475−1487.

(21) (a) Brandt, E. S.; Cotton, T. M. In *Investigations of Surfaces and Interfaces—Part B*, 2nd ed.; Rossiter, B. W., Baetzold, R. C., Eds.; John Wiley & Sons: New York, 1993; Vol. IXB, pp 633−717. (b) Cotton, T. M.; Kim, J.-H.; Chumanov, G. D. *J. Raman Spectrosc.* **1991**, *20*, 729−742. (c) Hobara, D.; Niki, K.; Zhou, C.; Chumanov, G.; Cotton, T. M. *Colloids Surf.* **1994**, *93*, 241−250.