$$L(\theta_r,\theta_i,\phi_r-\phi_i;\rho,\sigma) = \frac{\rho}{\pi}E_0 \cos\theta_i \times \{A + B\mathrm{Max}[0,\cos(\phi_r-\phi_i)]\, sin\alpha\, tan\beta\} \quad (1)$$

$$A = 1.0-0.5\frac{\sigma^2}{\sigma^2+0.33}$$

$$B = 0.45\frac{\sigma^2}{\sigma^2+0.09}$$

where E_0 is the intensity of the source, (θ_r, ϕ_r) and (θ_i, ϕ_i) are the observer and illuminant directions in a coordinate frame with its z axis aligned with the surface normal, and $\alpha = \mathrm{Max}(\theta_r, \theta_i)$ and $\beta = \mathrm{Min}(\theta_r, \theta_i)$.

Our model may be viewed as a generalization of Lambert's law, which is simply an extreme case with $\sigma = 0$. The model has direct implications for shape recovery in machine vision (*28*) and for realistic rendering in computer graphics (*29*). Further, it provides a firm basis for the study of visual perception of three-dimensional (3D) objects. To illustrate this, we compared digital images of several objects constructed from materials such as porcelain and stoneware with synthetic images of the objects rendered by using the model (Fig. 4). The images closely matched. Both real and rendered shadings vary synchronously, and significantly, with macroscopic roughness.

These experiments have led to a curious observation: Our model predicts that for very high macroscopic roughness, when the observer and the illuminant are close to one another, all surface normals will generate approximately the same brightness. This implies that a 3D object, irrespective of its shape, will produce nothing more than a silhouette with constant intensity within. In the case of polyhedra, edges between adjacent faces will no longer be discernible (Fig. 4A), and smoothly curved objects will be devoid of shading (Fig. 1A). This visual ambiguity may be viewed as a perceptual singularity in which interpretation of the 3D shape of an object from its image is impossible for both humans and machines. This phenomenon offers a plausible explanation for the flat-disc appearance of the full moon (Fig. 4E).

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27. Natural surfaces clearly profess a wide spectrum of macroscopic surface geometries. For deriving reflectance, a specific surface model must be assumed. In the past, several surface models (*19, 20*) have been found to be difficult to manipulate during reflectance model derivation. In the context of body reflection, radiance in the direction of the observer must be evaluated as an integral over all visible points on the surface patch projecting onto a single detector of the sensor. Because integration has the effect of weighted averaging, it turns out that several surface models are capable of capturing the primary reflectance characteristics of the surface. The V-cavity model (*21*) was chosen as it facilitates the analysis of pertinent radiometric and geometric phenomena and hence results in a reflectance model that performs fairly well for a large variety of real surfaces.
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Requirement of Carbon Dioxide for in Vitro Assembly of the Urease Nickel Metallocenter

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Assembly of protein metallocenters is not well understood. Urease offers a tractable system for examination of this process. Formation of the urease metallocenter in vivo is known to require four accessory proteins: UreD, postulated to be a urease-specific molecular chaperone; UreE, a nickel(II)-binding protein; and UreF and UreG, of unknown function. Activation of purified *Klebsiella aerogenes* urease apoprotein was accomplished in vitro by providing carbon dioxide (half-maximal activation at ~0.2 percent carbon dioxide) in addition to nickel ion. Activation coincided with carbon dioxide incorporation into urease in a pH-dependent reaction ($pK_a \geq 9$, where K_a is the acid constant). The concentration of carbon dioxide also affected the amount of activation of UreD-urease apoprotein complexes. These results suggest that carbon dioxide binding to urease apoprotein generates a ligand that facilitates productive nickel binding.

Urease, the first enzyme crystallized (*1*) and the first shown to include nickel (*2*), is found in certain plants, fungi, and bacteria. It participates in environmental nitrogen transformations and is a virulence factor in certain pathogenic microbes (*3*). According to x-ray absorption spectroscopic analysis (*4*), the protein contains a dinuclear Ni(II) active site (*5*) in which each metal atom has a Ni(imidazole)$_x$(N,O)$_{5-x}$ (x = 2 or 3) coordination environment. In vivo assembly of this metallocenter in *K. aerogenes* (*6*) involves the participation of four accessory gene products: UreD, UreE, UreF, and UreG (*7*).

UreD has been postulated to function as a molecular chaperone that stabilizes a urease apoprotein conformation that is competent for nickel incorporation (*8*). Evidence consistent with this hypothesis includes (i) our ability to purify several forms of a UreD-urease apoprotein complex of the formula (urease)$_3$UreD$_n$, where (urease)$_3$ is the native $(\alpha\beta\gamma)_3$ protein (n = 1, 2, or 3); (ii) the demonstration that these complexes can be partially activated by addition of nickel ions (increasing amounts of activation correlate to increasing n); and (iii) the finding that UreD dissociates from urease during activation. UreE has been proposed to serve as a

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nickel donor for urease activation (9) on the basis of its ability to bind approximately six nickel ions per dimer (dissociation constant $K_d \sim 10$ μM) with rather high specificity. The roles of UreF and UreG in urease activation are unknown, but they are absolutely essential for activation in vivo. UreG has been related in sequence to HypB, a guanosine 5′-triphosphate–binding protein that is required for incorporation of nickel into hydrogenase (hydrogen:acceptor oxidoreductase) (*10, 11*). Here, we demonstrate that CO_2 is required for in vitro activation of urease apoprotein. We detail several properties of this system, propose a mechanism for the in vitro process, and discuss how UreD may assist in urease activation.

Purified *K. aerogenes* urease apoprotein was activated in the absence of any accessory protein by incubation with nickel ion in the presence of bicarbonate-containing buffers (Fig. 1A). Because bicarbonate is in equilibrium with dissolved CO_2 in solution, it was imperative to determine which species is the actual activating factor. By using $NaHCO_3$ from stock solutions that were adjusted to pH 4.2 or 8.5 [the dissolved CO_2 concentration is much greater in the low-pH solution (*12*)], we demonstrated that urease activation was CO_2-dependent (Fig. 1B). Experiments in which carbonic anhydrase was included in the activation mixture provided additional support for this conclusion. The initial rapid activation (complete by ~5 min) observed in the low-pH $NaHCO_3$ solution was not observed in the presence of carbonic anhydrase; this result presumably was caused by the more rapid hydration of dissolved CO_2 at that pH (Fig. 1B). The bicarbonate (hence CO_2) concentration affected the extent of activation (Fig. 1A); the maximal specific activity (~310 U/mg) in this preparation accounted for activation of approximately 12.5% of the urease apoprotein, but activation of up to 30% was observed in another apoprotein preparation. Half-maximal activation of urease apoprotein at pH 8.3 occurred at approximately 10 mM bicarbonate, equivalent to about 0.063 mM or 0.2% CO_2 (*13*) [by comparison, the CO_2 concentration in the atmosphere is approximately 0.03% (*12*)]. Covalent incorporation of the activating CO_2 molecule into the enzyme was shown with the use of [^{14}C]$NaHCO_3$ (*14*). The radioactivity represented incorporation of 0.48 bicarbonate carbon atoms per αβγ unit. Because the specific activity generated in this experiment (241 U/mg) is much less than 48% of that expected for fully active enzyme (~2500 U/mg) (*15*), CO_2 incorporation does not equate to enzyme activation, and other factors must be important.

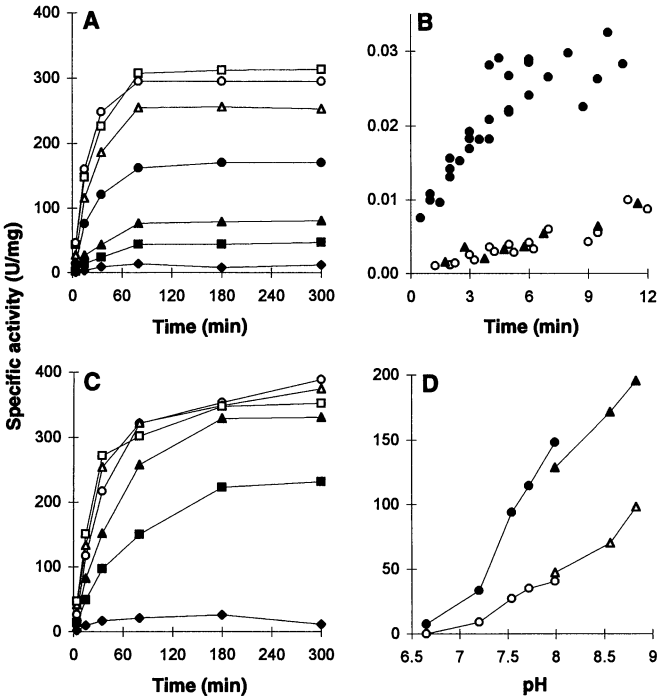

Fig. 1. In vitro activation of urease apoprotein. Urease apoprotein was purified from *Escherichia coli* DH5 cells bearing plasmid pKAU22Δ*ureD-1*, containing the *K. aerogenes* gene cluster deleted in *ureD* (*7*). Enzyme activity was assayed in 25 mM Hepes, 0.5 mM EDTA, and 50 mM urea (pH 7.75). The specific activity of urease was calculated from linear regression analysis of the rate of released ammonia, determined by conversion to indophenol (*22*), and from protein concentration (*23*). One unit of urease activity is defined as the amount of enzyme required to degrade 1 μmol of urea per minute at 37°C. (**A**) Apoprotein (final concentration of 0.8 mg/ml or 9.6 μM) was added to an activation buffer [100 mM Hepes (pH 8.3), 150 mM NaCl, and 10 μM EDTA] containing 100 μM $NiCl_2$ and 0 (◆), 1 (■), 3 (▲), 10 (●), 30 (△), 100 (□), or 200 (○) mM $NaHCO_3$ at 35°C. Samples were removed over time to monitor urease activity. The results are representative of four independent preparations. (**B**) Apoprotein (as above) was placed in an activation buffer containing 200 μM $NiCl_2$ at 0°C; $NaHCO_3$ was immediately added (final concentration of 1 mM) from stock solutions (4 mM) that were prepared at pH 4.2 (●) or 8.5 (▲), and timed samples were removed for assay. An identical low-pH $NaHCO_3$ stock solution experiment was carried out in the added presence of carbonic anhydrase (0.2 mg/ml) (○). The results are representative of three separate preparations. (**C**) Apoprotein (as above) was incubated at 35°C in an activation buffer containing 100 mM $NaHCO_3$, and $NiCl_2$ was added to yield final concentrations of 10 (◆), 20 (■), 30 (▲), 40 (○), 60 (△), or 100 (□) μM. Samples were removed and assayed at the indicated times. The results are representative of four independent experiments. (**D**) Activation buffers containing 100 mM Hepes (○ and ●) or 2-[*N*-cyclohexylamino]-ethanesulfonic acid (CHES) (△ and ▲), 100 μM $NiCl_2$, and carbonic anhydrase (0.2 mg/ml) (added to accelerate the approach to equilibrium) were incubated at 35°C in the presence of 0.3% CO_2 atmosphere. After a 30-min incubation, apoprotein (as above) was added and samples were removed for assay at 45 (○ and △) and 120 (● and ▲) min. The indicated pH values were measured at the conclusion of the experiment. The results are representative of two independent experiments.

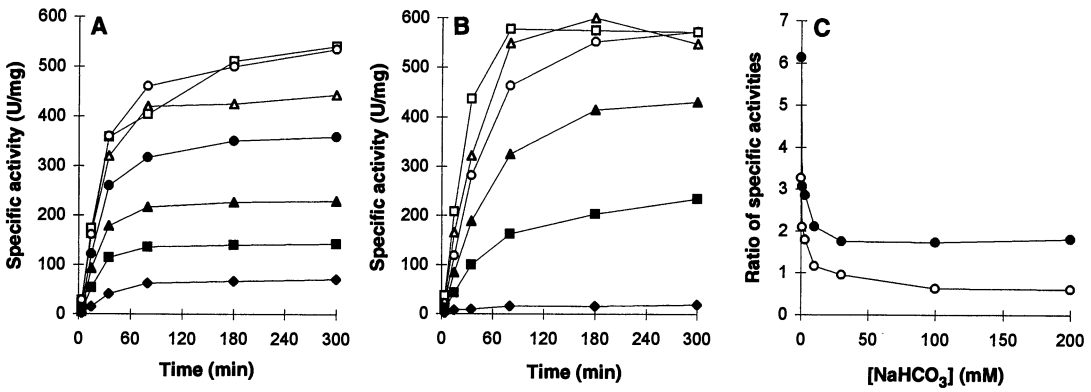

Fig. 2. In vitro activation of UreD-urease apoprotein complex. A mixture of UreD-urease apoprotein complexes was enriched through the Mono Q HR 10/10 step of purification (*8*). (**A**) The bicarbonate dependence of activation and (**B**) the nickel ion dependence of activation for the protein complex (0.8 mg/ml) was carried out as described for the apoprotein alone (Fig. 1, A and C, respectively). (**C**) The ratio of the specific activities that were generated during activation of the UreD-urease apoprotein and the urease apoprotein are compared as a function of bicarbonate concentration for 4 min (○) and 300 min (●) of activation.

The dependence of urease apoprotein activation on nickel ion concentration was assessed in buffer containing 100 mM $NaHCO_3$ (Fig. 1C). The activation rate appeared to saturate at approximately 60 μM nickel ion. This value is only about six times higher than the protein concentration, or only three times higher than the concentration needed to load the bi-nickel site of the protein. Prior incubation of urease apoprotein with nickel ion followed by addition of bicarbonate failed to yield active enzyme. This nonproductive interaction between apoprotein and nickel ion, which was reversible by prolonged incubation of the sample in the presence of EDTA, may account for the low extent of activation described above. Activation of urease apoprotein under an atmosphere of 0.3% CO_2 exhibited a pH dependence (Fig. 1D), with solutions of increasing pH leading to greater rates of activation. The pK_a of the activation process is 9 or greater; higher pH values could not be examined because of the excessive amounts of bicarbonate that would be present in the reaction mixture.

Urease activation kinetics, in which the extent of reaction was governed by the CO_2 concentration (Fig. 1A) and the rate was controlled by the nickel ion concentration (Fig. 1C), can be accommodated either by an interaction of CO_2 with apoprotein before metal ion binding or by formation of a metal ion–CO_2 complex that binds to the apoprotein. A well-characterized biological precedent for the former mechanism involves ribulose-1,5-bisphosphate carboxylase–oxygenase (RUBISCO). This magnesium-dependent enzyme has been shown to be activated by reaction of a lysine residue with CO_2, where the resulting carbamate serves as a metal ligand (*16*). A similar activation step may occur in 1-aminocyclopropane-1-carboxylate (ACC) oxidase, a ferrous ion–dependent enzyme (*17*). As we observed for urease, the activation rates for these enzymes are enhanced at higher pH values (*18*, *19*). We propose that metallocenter assembly into urease apoprotein occurs by reaction of a deprotonated protein side chain with an activating CO_2 molecule to generate a ligand that facilitates productive nickel binding. Unlike RUBISCO and ACC oxidase, however, urease activation was not reversed by addition of metal ion chelators. We speculate that urease activation may include a protein conformational change that serves to trap the metallocenter.

Our ability to partially activate urease apoprotein in the absence of any accessory protein compelled us to reevaluate the previous suggestion that UreD functions as a molecular chaperone (*8*). We therefore examined the effect of CO_2 on the activation of a mixture of UreD-urease apoprotein complexes of the formula $(urease)_3UreD_n$ (n = 0, 1, 2, or 3; $\bar{n} \approx 1.6$). The extent of activation depended on the bicarbonate concentration (Fig. 2A) and the rate depended on the nickel ion concentration (Fig. 2B), with concentration dependences and kinetics that closely resembled data obtained for urease apoprotein alone (Fig. 1, A and C). In two separate preparations, 0.51 or 0.36 carbon molecules were incorporated per αβγ unit from $[^{14}C]NaHCO_3$ treatment of the UreD-urease apoprotein complex mixture (*20*), again in agreement with the urease apoprotein studies. The incorporated radioactivity was not removed by incubation of the protein with unlabeled $NaHCO_3$ or urea, but it was lost by acid treatment (*20*). The final amount of activation for the UreD-urease apoprotein complex mixture was higher than for the urease apoprotein alone, although it was lower than expected on the basis of the amount of radioactivity incorporated; the effect was most pronounced at the lowest bicarbonate concentrations (Fig. 2C). Indeed, the indicated ratios for activation were underestimated by a factor of 1.19 if specific activity is measured in terms of units per milligram of urease protein rather than units per milligram of total protein for the experiments that include UreD (*21*).

To examine the effect of UreD on initial activation rates, we compared the activities (at 4 min) of the UreD-urease apoprotein complex and the apoprotein (Fig. 2C). The presence of UreD accelerated the rate at low bicarbonate concentrations, but at concentrations above ~50 mM the rate was reduced in the presence of UreD. Like the apoprotein, the UreD-urease apoprotein complex was shown to form a nonproductive nickel-urease species; however, the presence of UreD appeared to reduce the rate of this inactivation step. For example, when urease apoprotein was incubated for 10 min with 100 μM nickel ion before addition of bicarbonate, its ability to be activated was reduced to 8.8% of that where nickel ion and bicarbonate were provided simultaneously, whereas UreD-urease apoprotein complex that was treated in a similar manner retained 25% of its ability to be activated.

These results confirm the importance of UreD in urease activation at low concentrations of CO_2 and suggest a mechanism for this activation. We propose that UreD functions, at least in part, to minimize nonproductive binding of nickel to apoprotein that lacks bound CO_2—that is, to the protein species expected to predominate at low CO_2 concentrations. Hence UreD may still be considered to act as a molecular chaperone that controls the proper sequence of activation steps so that CO_2 binds before nickel ion.

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$$[CO_2] = \frac{C_T[H^+]^2}{K_{a1}K_{a2} + K_{a1}[H^+] + [H^+]^2}$$
where C_T is 10 mM, $[H^+]$ is 5.01×10^{-9} (for pH 8.3), and pK_{a1} and pK_{a2} are 6.0 and 9.74 [interpolated from tables 2.1 and 2.2 of (*12*)], respectively. Conversion from millimolar to percent concentrations used Henry's law, with pK_H estimated to be 1.61 (interpolated from the same source).
14. Urease apoprotein (final concentration of 0.5 mg/ml) was incubated in the standard activation buffer containing 100 μM $NiCl_2$ and 50 mM $NaHCO_3$ (1.44 mCi/mmol) for 9 hours, quenched with EDTA (final concentration of 5 mM), and chromatographed on a Mono Q 10/10 column (*15*). After chromatography, the enzyme specific activity was measured and the specific radioactivity was determined with a scintillation counter, with measurement of the stock $NaHCO_3$ radioactivity used as a control.
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21. To convert units per milligram of total protein to units per milligram of urease protein for the UreD-urease complexes, we multiplied the measured specific activity by M_r $(urease)_3UreD_n$ and divided by M_r $(urease)_3$, where M_r $(urease)_3$ is 249,300 and M_r UreD is 29,807.
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