Evidence for an elementary process in bone plasticity with an activation enthalpy of 1 eV

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The molecular mechanisms for plastic deformation of bone tissue are not well understood. We analysed temperature and strain-rate dependence of the tensile deformation behaviour in fibrolamellar bone, using a technique originally developed for studying plastic deformation in metals. We show that, beyond the elastic regime, bone is highly strain-rate sensitive, with an activation volume of ca. 0.6 nm³. We find an activation energy of 1.1 eV associated with the basic step involved in the plastic deformation of bone at the molecular level. This is much higher than the energy of hydrogen bonds, but it is lower than the energy required for breaking covalent bonds inside the collagen fibrils. Based on the magnitude of these quantities, we speculate that disruption of electrostatic bonds between polyelectrolyte molecules in the extracellular matrix of bone, perhaps mediated by polyvalent ions such as calcium, may be the rate-limiting elementary step in bone plasticity.

Keywords: bone plasticity; micromechanics of bone; deformation mechanisms; thermal activation; calcium mediated bonds

1. INTRODUCTION

Bone is fracture resistant and shows large plastic deformation (Rho et al. 1998; Fratzl et al. 2004). Little quantitative information is available on the nature of the basic molecular level rearrangements under stress, which make this irreversible plastic deformation possible. Plasticity has to do with breaking and rearrangement of bonds, and bone is not an exception. Such processes can be helped by thermal activation (Kocks et al. 1975). The question is which bonds are breaking and how. In the case of metals, for example, the elementary process is known to be associated with the movement of lattice dislocations (Kocks et al. 1975), a process not likely to occur in the protein–mineral composite bone. As plasticity is a major factor reducing bone fragility, its origin is of the highest interest, both for the fundamental understanding of biological composites as well as to assess the possible origin of age-related fracture (Zioupos 2001) occurring without apparent change in the overall mechanical properties (Zioupos & Currey 1998). Owing to the hierarchical structure of bone (Rho et al. 1998; Weiner & Wagner 1998), length-scales ranging from the tissue level at 1–100 μm (Nalla et al. 2003) to the molecular scale (Mercer et al. 2006) have been considered to be responsible for the inelastic behaviour of bone. Indeed, the majority of studies do not use plasticity concepts but rather damage models (Carter & Caler 1985; Schafferl et al. 1994; Zioupos & Currey 1994; Reilly & Currey 2000) to understand the post-yield behaviour in bone, where damage means phenomena, such as microcracking and microfractures (Zioupos & Currey 1994; Zioupos 1999), observed with confocal scanning and light microscopy techniques.

In general, plastic deformation corresponds to the opening and reforming of bonds, leading to a permanent deformation. Thermodynamically, this corresponds to a movement over local energy barriers at the molecular level—leading to creep on a macroscopic scale—and can be described as an Arrhenius-type rate process (Schoeck 1965; Gibbs 1967). For permanent plastic deformation at a stress σ and temperature T, the macroscopic strain rate dε/dt and the flow stress are related to each other through the microscopic activation energy barrier H and the volume v associated with the jump over the barrier, as

\[
\left( \frac{d\varepsilon}{dt} \right) = \left( \frac{d\varepsilon}{dt} \right)_0 \exp \left( -\frac{H - \sigma v}{k_B T} \right). \tag{1.1}
\]

The magnitude of H and v, thus obtained from macroscopic mechanical tests, give insight into the nature of the deformation mechanism at the molecular level. In metals and metal alloys, the activation volume for dislocation movement v can be written in terms of the Burgers vector for the basic dislocation step (Kocks et al. 1975), and thus provides information on the
nature of the dislocation mechanism (Caillard & Martin 2003). This motivates us to see whether we could, similarly, quantify experimentally the length-scale and energy barrier associated with the elementary step at the molecular level for plastic deformation in bone.

2. MATERIAL AND METHODS

Fibrolamellar bone from the periosteum of bovine femora (Gupta et al. 2005) was stretched to failure in a specially built tensile rig which enabled temperature control, from 4 to 50°C, in saline testing to keep the bone wet, and strain rates of up to 20–50% s⁻¹ measured with video extensometry (figure 1 and electronic supplementary material). The tensile specimens had a gauge length of 6 mm and a cross-sectional area of 0.08 mm² on average. Strain was measured from the percentage increase in separation of two markers on the bone imaged with a video camera. Temperature was typically kept constant during the test, although for a few measurements the temperature was changed abruptly in the yield region (see electronic supplementary material).

3. RESULTS

Activation volume: to measure the activation volume \( v \) of plastic deformation, bone samples were stretched at constant motor velocity into the plastic yield region as shown in figure 2. When the sample was clearly in the zone of plastic deformation, the strain rate was reduced either once or several times (figure 2, inset). This change led to a reduction of the flow stress but, interestingly, the slope of the post-yield curve (linear hardening \( d\sigma / d\varepsilon \)) remained constant. From equation (1.1), the activation volume can be estimated as

\[
v = k_B T \frac{d\ln(d\varepsilon / dt)}{d\sigma} |_{T}.
\]
Figure 2. Measurement of the activation volume $v$ in bone plasticity: the plot shows a uniaxial tensile test at constant temperature $T=296$ K with six stepwise reductions in motor velocity from 10 to 0.1 mm s$^{-1}$ ($a\to f\equiv10$ mm s$^{-1}\to5$ mm s$^{-1}\to2$ mm s$^{-1}\to1$ mm s$^{-1}\to0.5$ mm s$^{-1}\to0.2$ mm s$^{-1}\to0.1$ mm s$^{-1}$) and subsequent increases. The differential changes in stress with differential changes in strain rate can be used to compute the activation volume (equation (1.2)).

Using this differential method to estimate the activation volume leads to an average value of $v=1.00\pm 0.19$ nm$^3$ ($n=8$, error bars: standard deviations), implying that whatever (as yet unspecified) deformation processes occur in bone plasticity, the fundamental step is confined within a nanoscale volume. The activation volume is statistically independent ($p>0.05$) of stress, for a range of samples studied.

Activation enthalpy: to determine the height of the thermal activation barrier in bone deformation, numerous stretch-to-failure tests were done on bone tissue at strain rates varying from $1.4\times10^{-4}$ to $2\times10^{-1}$ s$^{-1}$ and temperatures from 4 to 37°C ($n=74$ total; see table 1 in the electronic supplementary material for breakdown by strain rate and temperature). The lowest strain rates used were comparable to those previously used to measure fibrillar deformation using synchrotron radiation (Gupta et al. 2005), the intermediate strain rate was comparable to the rates obtained from physiological in vivo measurements (Robertson & Smith 1978; Burr et al. 1996) and close to strain rates of 0.6 s$^{-1}$ typical for hip fractures (Courtney et al. 1996). The highest strain rates are a little below those rates at which the onset of brittle behaviour was observed (McElhaney 1966). For each sample, the yield stress $\sigma_Y$ was calculated as in figure 1a. Rewriting equation (1.1) as

$$\sigma_Y = \frac{1}{v} \left( H - X + Y \ln \left( \frac{dx}{dt} \right) \right), \quad \text{with}$$

$$X = k_B T \ln \left( \frac{dx}{dt} \right) \quad \text{and} \quad Y = k_B T,$$

we carried out a multiple linear regression of $\sigma_Y$ in terms of $X$ and $Y$. An extremely significant ($p<0.0001$) correlation was found between the dependent (stress $\sigma_Y$) and independent variables ($X$ and $Y$). The resulting fit parameters are: $H=1.11\pm 0.34$ eV; $v=0.64\pm 0.07$ nm$^3$; and $(dx/dt)_0=1.11\times10^5$ s$^{-1}$ $(3.00\times10^6\text{--}4.09\times10^{11}$ s$^{-1}$), and the plane fit is shown in figure 3a. Representing our data in terms of $\sigma_Y$--$T$ graphs, as usual in analyses of thermally activated plasticity (Kocks et al. 1975), we show mean value and standard deviation of the yield stress for several values of temperature and strain rate in figure 3b. The yield stress decreases with increasing temperature for all the three strain rates. The broken lines show how equation (1.3) predicts that the yield stress $\sigma_Y$ would vary as a

Figure 3. Measurement of the activation enthalpy $H$ of bone plasticity: a set of samples ($n=74$) are stretched to failure in tension, at three strain rates and at least three temperatures at each strain rate, from 277 to 310 K, and the yield stress $\sigma_Y$ measured. Plot (a) shows the yield stress $\sigma_Y$ as a function of $k_BT$ and $k_BT\ln(dx/dt)$. Black grid lines show the results of the linear plane fit $\sigma_Y(T;dx/dt)=(H/v)+(1/v)k_BT\ln(dx/dt)=(\ln(dx/dt)_0/v)k_BT$. Plot (b) shows the same three-dimensional plot in the $\sigma_Y$--$k_BT$ plane, giving the average stress (error bars: standard deviations) for a given strain rate and temperature. Lines are predictions based on model fit in (a), at the given three strain rates: dash-dotted lines and white circles $\equiv2.2\times10^{-1}$ s$^{-1}$; short dashed lines and grey symbols $\equiv5.4\times10^{-3}$ s$^{-1}$; solid lines and black symbols $\equiv1.4\times10^{-4}$ s$^{-1}$. Note that the fact that we view (a) at an angle almost 90° to the effective viewing direction in (b) is deliberate, chosen to give the reader the perspective from two orthogonal directions.

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function of temperature at a given strain rate (using the fitted parameters from figure 3a).

The discrepancy between the activation volumes obtained from the global survey of yield stress data (0.64 ± 0.07 nm$^3$) and from the differential data of various flow stresses (1.00 ± 0.19 nm$^3$) is not surprising. The former refer to specimens with unmodified microstructure, at the onset of plastic deformation, the latter to microstructures already modified by plastic deformation. Such subtleties amount to strain and stress dependence of the activation volume (Kocks et al. 1975) and are beyond the concern of present bone research. We therefore take the latter value as confirmation of the former.

4. DISCUSSION

To summarize, we find that plastic deformation in bone is characterized by a very small activation volume $v$ of the order of 1 nm$^3$ and an activation enthalpy of the order of $H = 1.1$ eV. This activation enthalpy is smaller than typical covalent bond energies (C–C bond approx. 3.6 eV) but much larger than hydrogen bonds (approx. 40 meV). The Gibbs free energy $G(\sigma) = H - v\sigma$ is the free energy to be supplied during one activation event in the plastic deformation of bone. The applied stress $\sigma$ increases the probability of the irreversible deformation occurring, by doing work against a certain basic volume of deformation $v$. $H$ and $v$ carry information on the energy barrier needed to go from the undeformed to the deformed state, but they give no information about the kinetics of this process.

The small activation volume suggests that the elementary process corresponds to the breaking of just a few spatially confined bonds. In metal plasticity, which is controlled by the dynamics of dislocations (Schoeck 1965; Gibbs 1967; Kocks et al. 1975), the activation volume is the area of slip $s$ x the Burgers vector (Kirchner 2006). In the case of bone, the small size of the activation volume is likely owing to a confinement of the soft organic matrix (which is likely to flow) between nanometre-sized particles. Size effects on mechanical properties are well known from the science of materials strength as seen, for example, in recent work (Uchic et al. 2004; Espinosa et al. 2005).

Our recent in situ diffraction results (Gupta et al. 2005, 2006) suggest that after the onset of macroscopic plasticity, only elastic deformation is retained within fibrils and plastic deformation occurs between them.

The magnitude of the activation enthalpy $H = 1.1$ eV suggests that the bonds being broken are not likely covalent. Hydrogen bonds, having energy of 40 meV each, would have to break in large numbers (approx. 50) simultaneously to provide the necessary energy, but such a situation is inconsistent with the small activation volume of up to 1 nm$^3$. As a consequence, the most likely types of bonds are charge interactions between molecules in the extracellular space. It is not known which these molecules are, but substantial amounts of non-collagenous molecules, such as proteoglycans (Scott 1992), osteopontin (Sodek et al. 2000) or fetuin A (Heiss et al. 2003), are present in the bone matrix. These (mostly negatively) charged molecules (or any combination of them) could be responsible for forming a plastic ‘glue’ between fibrils. The existence of such a ‘glue’ has recently been proposed following force spectroscopy experiments (Thompson et al. 2001) and it was shown that the occurrence of bond breaking and reforming was related to the presence of calcium ions (Fantner et al. 2005, 2006). The energy associated with these ‘sacrificial bonds’ is consistent with the activation enthalpy of 1 eV found here (Fantner et al. 2006).

Recently, it has also been shown that the deformation of polyelectrolyte capsules is associated with breaking a group of neighbouring charge interactions on polyelectrolyte segment (Leporatti et al. 2001). The activation enthalpy was shown to be ca 1 eV in this case and the activation volume (corresponding to the size of the polyelectrolyte segment) was also in the range of ca 1 nm$^3$ (Leporatti et al. 2001). Breaking and reformation of bonds has also been found in the hemicellulose matrix between cellulose fibrils in the cell wall during plastic deformation of wood (Keckes et al. 2003).

Thermodynamics of plastic deformation interprets the pre-exponential factor $(de/dt)|_0$ as a product of an attempt frequency $v_0$ and the deformation $v_0$ caused by each activated event. The latter quantity $v_0$ depends on the process controlling strain (dislocation density, obstacle density, etc.). It is difficult to put a precise value for the attack frequency $v_0$, but it must be of the order of vibrations present in the medium. The phonon spectrum of bone has never been measured, but presumably, it must be similar to the spectrum of type I collagen found in tendon (Middendorf et al. 1995). The latter shows several broad maxima between 1 $\times$ 10$^{13}$ and 6 $\times$ 10$^{13}$ s$^{-1}$, which have been attributed to various localized modes. Given the fact that possible localized modes at the interface between fibrils must be low-frequency ones otherwise the interfacial entropy would be negative, it is not unreasonable to assume a value of $v_0 = 10^{12-13}$ s$^{-1}$. Our fit results provide a value lower than this range (1.11 $\times$ 10$^{10}$ s$^{-1}$) but, unsurprisingly in light of the discussion above, this is the fit parameter that showed a substantial error (approx. 30% in the logarithm) in the multiple linear regression, leading to a possible range of values from $3.00 \times 10^6$ to 4.09 $\times 10^{13}$ s$^{-1}$.

Damage has been associated with the post-yield behaviour, but the nature of the damage is unclear (Carter & Caler 1985; Schaffler et al. 1994; Zioupos & Currey 1994; Zioupos et al. 1994; Zioupos 1999; Reilly & Currey 2000). The damage is believed to be related to the formation of microcracks or smaller defects at weak interfaces such as between old and new bone packets in trabecular bone, between lamellae in lamellar cortical bone or between osteons and interstitial bone (Lakes & Saha 1979; Braidotti et al. 2000; Diab et al. 2006; Peterlik et al. 2006). In the present work, we try to avoid the effect of these weak interfaces, both by preparing samples whose cross-section is of the order of the width of single fibrolamellar bone packets (see electronic supplementary material) and by studying this relatively parallel fibre tissue in tension. Nevertheless, the breaking of bonds (within an activation volume of ca 1 nm$^3$) can be regarded as damage at the supramolecular level which has, however, the
In conclusion, our mechanical tests on bone established a high sensitivity of the macroscopic plastic deformation to the strain rate and temperature. By putting our results in the scheme of thermally activated processes controlling bone plasticity, quantitative results can be obtained on the length-scale and energy associated with bone plasticity mechanisms at the molecular level. The fundamental processes involved in plastic deformation are localized to within 1 nm³, and with energy of the order of 1 eV. We speculate that these processes are localized in a small fraction of the bone tissue—the extrafibrillar matrix—and correspond to the disruption of calcium-mediated ionic bonds between the long and irregular chains of molecules constituting this matrix.

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