adjacent chains sticking slightly to one another.) The combination of the two contributions was evaluated by Tarasov^{6,7} involving effective Debye temperatures θ_1 (one dimensional) and θ_3 (three dimensional), which give a measure of the appropriate one and three dimensional elastic force constants in an assembly of polymeric molecules.

We have measured the low temperature specific heat of an apparently random sample of poly(L-alanine) (PLA, Pilot Division of New England Nuclear Corp., Mass.), that is, poly(-NH-CHR-CO-) with $R=CH_3$, which is structurally the simplest homopolypeptide after poly(glycine) ($R=H$). The structurally simplest polypeptides, particularly those of high molecular weight, are insoluble in commonly used reagents, leading to many difficulties in analysis. The limiting viscosity number of PLA in dichloroacetic acid was 68 ± 2 ml./g (E. K. Lai, personal communication) implying (from poly-DL-alanine)⁸ a molecular weight of approximately 19,000 or a degree of polymerization of 270. Preliminary ultracentrifuge measurements by Professor W. R. Bauer (Department of Chemistry, Colorado University) suggest that the PLA is quite poly-disperse in molecular weight. Low angle (up to 3°) X-ray scattering measurements by Dr D. H. Reneker (Polymer Crystal Physics Division, National Bureau of Standards) show no fibre or crystalline structure, and are typical of those of amorphous polymers. Infrared absorption measurements ($400\text{--}4,000\text{ cm}^{-1}$) give no evidence of alpha (helix) or beta (sheet) structure⁹.

To measure specific heat, C , one applies an amount of heat ΔQ to a thermally insulated sample and measures the resulting temperature rise ΔT . Then $C = \Delta Q / \Delta T$. The specific heat apparatus is described elsewhere¹¹. The ratio (heat capacity of PLA)/(heat capacity of holder, copper and thermometer) was 1.9 at 2 K, 3.5 at 10 K and 1.7 at 20 K.

Fig. 1 Specific heat (divided by temperature cubed, for scaling) of random poly(L-alanine) 1–10 K. The broken curve is a fit to the Tarasov model; the full curve is a fit to the Tarasov model with one Einstein mode.

The results above 10 K are easily fitted to the model^{6,7}, showing definite one dimensional and three dimensional molecular contributions to the specific heat. The gram-molecular weight of the vibrating unit was taken as 71.08. At lower temperatures, the experimental data (Fig. 1) give evidence for another contribution to C . Such anomalies have been observed in other polymers and glasses^{11–15}, and have been accounted for by assuming that a small mole-fraction, f , of the total vibrational modes are Einstein oscillators of characteristic temperature θ_E ^{11,12} (possibly due to lattice resonant modes^{11,12}). The corresponding fit (Tarasov model plus Einstein oscillator) for our data (Fig. 1) is quite good (s.d. = 3.6%) giving parameters

$$\theta_1 = 323\text{ K (2.4\%); } \theta_3 = 54\text{ K (4.2\%); } \theta_E = 12.4\text{ K (1.2\%); } f = 0.0032\text{ (0.4\%).}$$

The numbers (x%) are the change in the s.d. of the total specific heat fit when a parameter value is increased by 5% from the values quoted.

Lattice dynamical calculations of C have been made for alpha-PLA^{16,17}. No similar studies of amorphous PLA—which is a solid state equivalent of the random coil in the helix-coil transition—seem to have been made, although related problems^{18–20} have been treated.

These results illustrate that specific heat measurements at low temperatures of polypeptides can be useful for the measurement of molecular elasticity. The interpretation will be valid at room temperature (provided there are no unexpected conformational changes). Comparisons can be made with vibrational spectra from theoretical calculations. This work has obvious extensions to other biopolymers.

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Note added in proof. We have extended this work to predominantly alpha and beta PLA (*Nature* and *Biopolymers*, in the press). P. Delhaes, M. Daurel and E. Dupart have done similar work (*C R Acad. Sci. Paris*, in the press).

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Is "Orbital Steering" a New Concept?

INTRAMOLECULAR reactions have, for many years, been used as models for the productive ES complex in enzymatic reactions^{1–3}. Chemists have sought explanations for the rapid rates in certain intramolecular reactions hoping that, by understanding these factors, much could be gained towards the understanding of the efficiency of enzymatic catalysis.

Table 1 Relative Rates of Anhydride and Lactone (Ester) Formation

	Anhydride formation ⁹	k_{rel}	Lactone (ester) formation ⁴	k_{rel}
1a	$CH_3COO^- + CH_3COOC_6H_4Br(p)$	1.0	1b $CH_3COOH + EtOH$	1.0
2a		$\sim 1 \times 10^3$ M	2b	0.79×10^2 M
3a		$\sim 2.2 \times 10^5$ M	3b	6.6×10^3 M
4a		$\sim 5 \times 10^7$ M	4b	1×10^6 M

The term "orbital steering" was introduced by Koshland and co-workers to convey the concept that much of the facility of enzymatic reactions can be explained on the basis that the active sites of enzymes are so constructed that they align ("steer") the orbitals of substrate and catalytic groups optimally to enter the transition state^{4,5}. Without questioning the validity of this concept⁶ or the assumptions made by Koshland^{7,8} and co-workers in their quasi-theoretical treatment, what new factors are introduced by "orbital steering" to the arguments concerning steric acceleration in intramolecular reactions and, by implication, into enzymatic catalysis as well? If one examines experimental results it is obvious that "orbital steering" is a term provided for a well known hypothesis¹⁻³ (Table 1). On the right hand side of Table 1 are the experimental results of Storm and Koshland⁴ which were used to substantiate the importance of "orbital steering"; on the left hand side are the results of Bruice and Pandit⁹. The similarity of the experimental approach is most remarkable as are the similarities in rate enhancements. From the conclusion of the Bruice-Pandit paper: "These results point to the tremendous enhancement of rate that an enzyme could achieve by fixing the reacting species in a steric conformation closely resembling that of the transition state for the reaction", meaning that interacting orbitals must be aligned. Rather than providing a new concept in biochemistry, the study of Storm and Koshland is more an application of an existing experimental approach to provide an interesting but additional example.

The present controversy surrounding the explanation of the facility of enzymatic catalysis revolves around the quantitative importance of three proposals. The first two involve ground state phenomena and the third transition state stabilization. They are: (1) the free energy of the ground state is sufficiently raised by bringing together (elimination of translational freedom) and sterically aligning substrate and active site functional groups to enter the transition state; (2) the ground state free energy is sufficiently raised from loss of translational and rotational entropy on formation of a tight productive ES complex⁶; and (3) the transition state free energy is sufficiently lowered due to preferential binding of transition over ground state¹⁰.

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Cyanate Inhibition of Erythrocyte Glucose-6-phosphate Dehydrogenase

It has been suggested that cyanate (CNO) may be useful as a therapeutic agent in the prevention of occlusive sickle cell crises¹. Cyanate irreversibly carbamylates the N-terminal amino (NH₂) groups of proteins and the free epsilon -NH₂ groups of lysine². Carbamylation of the terminal valine residue of sickle haemoglobin is thought to be responsible for preventing affected red blood cells (RBCs) from sickling when they are deoxygenated¹. Sick cell erythrocytes incubated with 50 mM cyanate for 90 min *in vitro* reportedly have an increased survival when they are radiolabelled and reinfused into the donor³. This report describes a possible side-effect of RBCs treated *in vitro* with sodium cyanate in similar experimental conditions: there is irreversible inactivation of the enzyme glucose-6-phosphate dehydrogenase (G6PD).

Erythrocytes from healthy human adults were collected in EDTA and washed three times with a pH 7.4 phosphate-buffered salt solution (PBS) containing 110 mM NaCl, 20 mM Na₂HPO₄, and 4 mM KH₂PO₄. After the final wash, a 20% RBC suspension was made, and glucose added to a final concentration of 10 mM. When neutralized sodium cyanate was used, an equivalent amount of chloride was withheld to maintain a constant anionic strength. Aliquots of the reconstituted erythrocyte suspension were incubated at 37° C in a Dubnoff metabolic shaker at 100 oscillations per minute. Established methods were used to measure reduced glutathione (GSH), oxidized glutathione (GSSG), and G6PD⁴. Hexosemonophosphate (HMP) shunt activity was studied by measuring the oxidation of glucose-1-¹⁴C to ¹⁴CO₂ (ref. 5). This pathway was evaluated under resting conditions, during a peroxide stress with 20 mM sodium ascorbate, and with 0.2 mM methylene blue. Ascorbate produces H₂O₂ after reacting with oxyhaemoglobin. Methylene blue transfers electrons from dihydronicotinamide adenine dinucleotide phosphate (NADPH) to oxygen. Both of these reactions increase nicotinamide adenine dinucleotide diphosphate (NADP) availability, and thereby stimulate the HMP shunt (Fig. 1)^{6,7}. GSH stability was determined by measuring the changes in