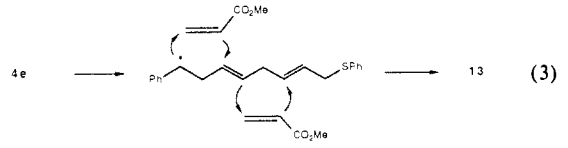
constant for bimolecular addition. The stereochemical outcome of the [3 + 2] addition process, which is determined during closure of the substituted 5-hexenyl radical **15** (via conformers **18** or **19**), can be rationalized by modifying existing models for such cyclizations.[12]

The cyclopropylcyclopropane substrate **4e** was examined in an effort to exploit the chain reaction nature of this process. Thus, serial double alkene addition within the propagation portion of the reaction sequence affords bis cyclopentane **13**, isolated as a mixture of at least five (^{1}H NMR) isomers (eq 3). Despite the lack of stereocontrol, the regioselective formation of four carbon–carbon bonds in a single transformation demonstrates the potential of this strategy for the efficient construction of polycyclic systems.



(3)

The role that the Lewis acid plays in these free radical reactions is obscure at present. Generally, we have observed that trimethylaluminum increases the rate and improves the stereoselectivity of these free-radical-mediated [3 atom + 2 atom] additions. Application of this methodology to the stereoselective synthesis of substituted cyclopentane natural products is in progress.

Acknowledgment. We thank the National Institutes of Health (GM 37681) for financial support.

Supplementary Material Available: Spectral data for **6–13** (7 pages). Ordering information is given on any current masthead page.

(11) Estimated from the rate constant for addition of 2-propionitrile radical to vinylene carbonate.[10b]

(12) Experimental: (a) Beckwith, A. L. J.; Easton, C. J.; Serelis, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 482. (b) Beckwith, A. L. J.; Lawrence, T.; Serelis, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 484, and references cited therein. Computational: (c) Spellmeyer, D. C.; Houk, K. N. *J. Org. Chem.* **1987**, *52*, 959. (d) Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron Lett.* **1985**, *26*, 373. (e) Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron* **1985**, *41*, 3925.

Electrophilic Catalysis by Vanadate of the Dehydration of Hydrated Glyoxylate

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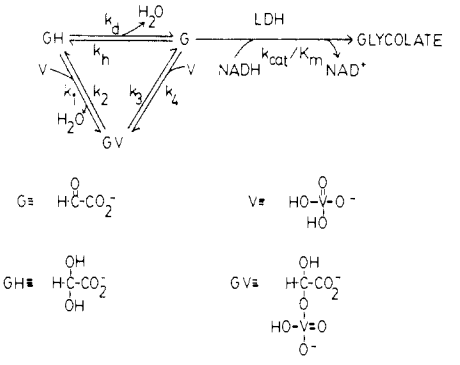
Vanadate was found to catalyze the dehydration of hydrated glyoxylate, and the results are interpreted in terms of Scheme I. In this model inorganic vanadate (V) condenses with one of the hydroxyl groups of the hydrated aldehyde (GH) to form a vanadate ester (GV), which eliminates vanadate to form the free aldehyde (G).

The dehydration rate was measured by utilizing the fact that only the free aldehyde is a substrate for lactate dehydrogenase (LDH)[1] and following the oxidation of NADH spectrophotometrically at 340 nm, by using an extinction coefficient of 6.22×10^3. As the concentration of LDH was increased, the reaction rate increased and approached a maximum as shown in Figure 1. This saturation behavior is attributed to the dehydration reaction becoming the rate-limiting step in the process. The rate increase in the presence of vanadate cannot reasonably be ascribed to general acid–base catalysis since similar concentrations of phosphate or arsenate did not observably affect the rate between pH 6 and 8.5, while vanadate increased the rate throughout this pH range.

Scheme I. The Uncatalyzed Reversible Hydration of Glyoxylate and an Alternate Pathway Which Proceeds via Formation of a Vanadate Ester[a]



[a] The free aldehyde is trapped by the lactate dehydrogenase-catalyzed reduction to glycolate.

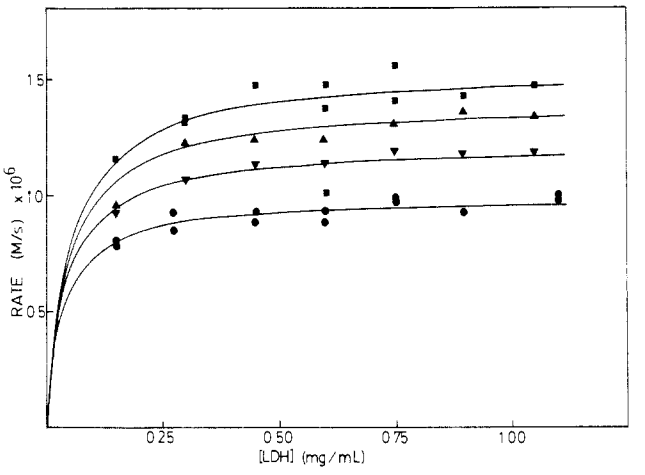


Figure 1. The effect of lactate dehydrogenase concentration on the rate of reduction of glyoxylate in the presence and absence of vanadate. Conditions were the following: 0.1 M HEPES, pH 7.2, 25 °C, 0.15 mM NADH, 0.1 mM glyoxylic acid, and the indicated concentrations of LDH (type XI from Sigma). Reactions were initiated by the addition of glyoxylic acid. Total vanadium atom concentrations (added as inorganic vanadate) were as follows: (●), 0 (▼) 0.2, (▲) 0.4, (■) 0.6 mM. The solid lines were calculated from eq 4 by using the constants $k_d = 0.0098$ s^{-1}, $k_3K_{eq} = 12.66$ M^{-1}·s^{-1}, $k_h/(k_{cat}/K_m) = 0.0336$ mg·mL^{-1}, and $k_4/(k_{cat}/K_m) = 36$ mg·mL^{-1}·M^{-1}. The values used in the calculations for vanadate concentrations were (▼) 0.179, (▲) 0.322, and (■) 0.437 mM.

The catalytic mechanism shown in Scheme I was suggested by the observations that vanadate esters form rapidly[2-4] and that elimination of phosphate from the phosphorylated hydrate of D-glyceraldehyde is several times faster than dehydration of the hydrate under similar conditions.[5]

The results shown in Figure 1 were analyzed as follows. Assuming that the vanadate ester formation is at equilibrium, which is reasonable in view of the rapid reversible formation of ethyl vanadate, results in eq 1; the fact that the concentration of free aldehyde is much below its K_m as a substrate for LDH[6] yields eq 2; and eq 3 results from applying the steady-state approximation to the free aldehyde. These equations yield eq 4.

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$$K_{eq} = \frac{k_1}{k_2} = \frac{[GV]}{[GH][V]} \quad (1)$$

$$R = \frac{d[NAD^+]}{dt} = \frac{k_{cat}}{K_m}[G][LDH] \quad (2)$$

$$[G] = \frac{[GH](k_d + k_3K_{eq}[V])}{k_h + k_4[V] + \frac{k_{cat}}{K_m}[LDH]} \quad (3)$$

$$\frac{1}{R} = \frac{1}{[LDH]}\left[\frac{k_h + k_4[V]}{[GH]\frac{k_{cat}}{K_m}(k_d + k_3K_{eq}[V])}\right] + \frac{1}{[GH](k_d + k_3K_{eq}[V])} \quad (4)$$

It is assumed that [GH] is essentially equal to the total glyoxylate concentration since $k_h/k_d > 100$,[7,8] and it is expected, by analogy with the case of esterification by vanadate of the hydroxyl group of lactate,[4] that $K_{eq}[V] < 0.01$. The concentrations of monomeric vanadate, [V], were calculated from the vanadium atom concentrations and the equilibrium constants for formation of vanadate oligomers, as described elsewhere.[9] Initial estimates for the parameters of eq 4 were obtained from appropriate plots of the data shown in Figure 1, and then a nonlinear least-squares program (BMDP) was used to fit eq 4 to all of the data shown in Figure 1. The values thus obtained were as follows: $k_d = 0.0098 \pm 0.0002$ s^{-1}, $k_3K_{eq} = 12.7 \pm 0.8$ M^{-1}·s^{-1}, $k_h/(k_{cat}/K_m) = 0.034 \pm 0.009$ mg·mL^{-1}, $k_4/(k_{cat}/K_m) = 36 \pm 28$ mg·mL^{-1}·M^{-1}. Similar values were obtained when the experiment was repeated by using lower concentrations of [LDH], and the values were not affected by changing the concentration of glyoxylate.

The value obtained for k_d is similar to that obtained by other investigators under similar conditions with the same experimental method.[7] From the value of k_3K_{eq}, an estimate can be made for k_3 if it is assumed that the value of K_{eq} is similar to the corresponding value obtained for lactate. The value of K_{eq} for lactate, 0.5 M^{-1}, was obtained at 1.0 M ionic strength, pH 7.35, but it is a reasonable first approximation to use for K_{eq} in Scheme I. By using a value of 1.0 M^{-1} for K_{eq}, to take into account the presence of two hydroxyl groups on GH, $k_3 = 12.7$ s^{-1}. This value is more than 10^2 times that reported for the elimination of phosphate from the phosphorylated hydrate of glyceraldehyde at 15°, pH 7.[5] This difference could be due to the negatively charged carboxylate group on glyoxylate or possibly to the ease with which the V–O bonds can undergo rehydridization upon elimination of vanadate. It is also possible that the reactive species GV is a complex in which GH acts as a bidentate ligand in the same way that lactate can.[4] Similar cyclic complexes have been proposed to rationalize catalysis of other dehydration reactions by transition metals.[10,11]

By using the published value of 163 for k_h/k_d at 25°, pH 7.4[8] as an approximation for k_h/k_d under the conditions used here, and the value determined above for k_d, the value of k_h can be estimated at 1.60 s^{-1}, and $k_4 = k_3k_{eq}k_h/k_d = 2.1 \times 10^3$ M^{-1}·s^{-1}.

We conclude that vanadate is an electrophilic catalyst of aldehyde dehydration and a nucleophilic catalyst of aldehyde hydration. Since there is evidence that oxalyl thioesters, formed from glyoxylate, act as intracellular messengers for insulin and some other hormones,[8,12] it is reasonable to consider whether the type of chemistry reported here might be responsible for some of the physiological effects of vanadium.[13,14]

Acknowledgment. This work was supported by a grant from the Medical Research Council, Canada.

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(15) Abbreviations: LDH, L lactate; NAD oxidoreductase, (EC 1.1.27); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADH, β-nicotinamide adenine dinucleotide, reduced form; NAD, β-nicotinamide adenine dinucleotide.

Chemical Potential Driven Contraction and Relaxation by Ionic Strength Modulation of an Inverse Temperature Transition

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The sequential polypentapeptide of elastin,[1,2] (L-Val^1-L-Pro^2-Gly^3-L-Val^4-Gly^5)$_n$, when cross-linked by γ-irradiation and when in equilibrium with water undergoes a reversible contraction on raising the temperature from 20 °C to 40 °C.[3] That this is the result of an inverse temperature transition has been shown by many physical characterizations[4] and is also evidenced by observing in water that analogues which are more hydrophobic undergo the transition at lower temperatures,[5] whereas less hydrophobic analogues undergo the transition at higher temperatures.[6] In this communication, it is demonstrated that a change in salt concentration causes a shift in the temperature of the inverse temperature transition and in particular that contraction and relaxation can be achieved by such changes in ionic strength. To our knowledge, this is the first demonstration that changes in chemical potential can produce contraction and relaxation in a neutral polymer and in particular in a synthetic polypeptide containing only aliphatic (Val and Pro) or no (Gly) side chains where the process is one of ionic strength modulation of an inverse temperature transition.

The polypentapeptide was synthesized as previously described.[7,8] This material is soluble in water in all proportions below 25 °C but on raising the temperature aggregation occurs.[9] Aggregation may be monitored by following the temperature dependence of solution turbidity as shown in Figure 1A for water and for phosphate buffered saline (0.15 N NaCl, 0.01 M phosphate) which is the physiological buffer system. In Figure 1A it is seen that phosphate buffered saline (PBS) causes aggregation to begin at a lower temperature while the effect of pH, curves a and b Figure 1A, is minimal, almost within the reproducibility of the data. The aggregates settle to form a more dense phase called the coacervate which in water is 38% peptide and 62% water by weight at 40 °C.[9] The coacervate is a viscoelastic phase which can be formed

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