(13) We are very grateful to Professor E. Piers for a generous sample of (±)-norseychellenone.

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Nuclear Magnetic Resonance Manifestation of the Configurational Differences between the L- and D-Norepinephrine Complexes with Cobaltous Adenosine 5'-Triphosphate, an Aqueous Chiral Shift Reagent

Sir:

Enantiomers in achiral environments give rise to identical NMR spectra. Spectral resolution of enantiomers has been achieved by employing chiral solvents[1] or by applying chiral paramagnetic lanthanide shift reagents.[2] Hitherto these methods have been restricted to nonaqueous media. ATP (and its metal chelates) contains the chiral D-ribose moiety and upon association with enantiomers should provide the chiral environment necsssary for spectral resolution. However, the chemical shifts induced by ATP in the protons of catecholamines are rather small, 0.3 ppm or less.[3] Thus the addition of ATP to the racemic mixture of norepinephrine, $(OH)_2$-$C_6H_3C_\beta HOHC_\alpha H_2NH_3^+$, resulted in measurable shifts for all of the protons similar to those previously obtained with the pure L enantiomer[3] but no spectral resolution into enantiomers was observed. Analysis of the chemical-shift data yielded formation constants for association with ATP and intrinsic shifts in the complexed state similar to those for pure L-norepinephrine[3] indicating that within the limits of resolution both enantiomers are equally complexed. This observation is in accord with the finding that complexes of catecholamines with ATP are stabilized mainly by ring stacking and by electrostatic interaction between the ammonium and phosphate groups[3,4] and suggests that these interactions are present in the ATP complexes of both of the norepinephrine enantiomers. It is reasonable to assume, therefore, that the gross structures of the complexes are similar. In view of the structural model proposed for the ATP complexes of catecholamines,[5] this similarity obviously implies different dispositions of only the substituents on the β-carbon atom relative to the ATP molecule. Schematic models of the structures of the ATP complexes with L- and D-norepinephrine are shown in Figure 1. On the basis of these models spectral resolution of enantiomers is expected for the β proton. Unfortunately this proton is not only subject to relatively small induced shifts but its resonance is obscured by the residual HDO signal of the solvent.

The chelation of a divalent metal ion by ATP results only in a slight reduction in the complexing ability of the latter for catecholamines and in minor alteration of the structure of the catecholamine–ATP complex, while offering the advantage of large dipolar shifts when Co^{2+} is the cation.[4-6] The effect of the cobaltous ATP chelate on the aliphatic portion of the proton NMR spectrum of racemic norepinephrine is shown in Figure 2, where for comparison the spectrum of L-norepinephrine taken under similar conditions is also given. It is seen that two sets of resonances are observed with the racemic mixture: one for each enantiomer. No separation into enantiomeric signals was observed in the aromatic portion of the spectrum. At the lower temperature both the extent of association and the intrinsic shifts are enhanced resulting in higher resolution. Although the induced shift in the β proton is not the largest, it exhibits the highest dispersion as anticipated from
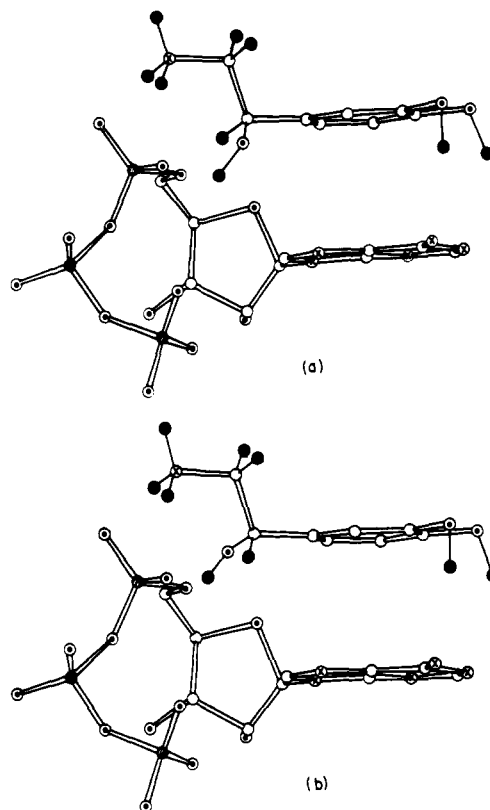
Figure 1. Schematic representation (side view) of the preferred structures of the ATP complexes with (a) L-norepinephrine and (b) D-norepinephrine.

Table I. Chemical Shifts[a] Relative to the Uncomplexed State of the 1:1 Complexes of L- and D-Norepinephrine with CoATP at 27 °C

proton	L-NE	D-NE
H_2, ring	2.09	2.09
H_5, ring	2.09	2.09
H_6, ring	3.05	3.05
H_β	4.40	3.49
H_α	4.26	4.66
$H_{\alpha'}$	5.96	5.96

[a] In parts per million.

the structural models (cf. Figure 1). Since similar concentrations were employed for both samples, the facts that the protons of the L enantiomer experience the same chemical shifts when taken alone or in the racemic mixture (compare traces a and b in Figure 2) and that the aromatic protons remain unseparated indicate that the complex formation constants for the two enantiomers are the same. A formation constant of 15 ± 3 M^{-1} at 27 °C was determined from titrations of fixed norepinephrine concentrations with CoATP in a manner similar to that previously described.[5] The chemical shifts (upfield relative to the uncomplexed state) of the norepinephrine–CoATP complexes resulting from the data analysis are summarized in Table I. The line width of the β proton in the complexed state is 21 Hz for L-norepinephrine and 13 Hz for the D enantiomer. The former value is in good agreement with the value of 19.8 Hz calculated from the longitudinal relaxation rate,[5] indicating that the line width is governed by the dipolar interaction with the Co^{2+} ion. A distance of 4.9 Å between the β proton of L-noreinephrine and the cobaltous ion of CoATP has previously been determined from the relationship
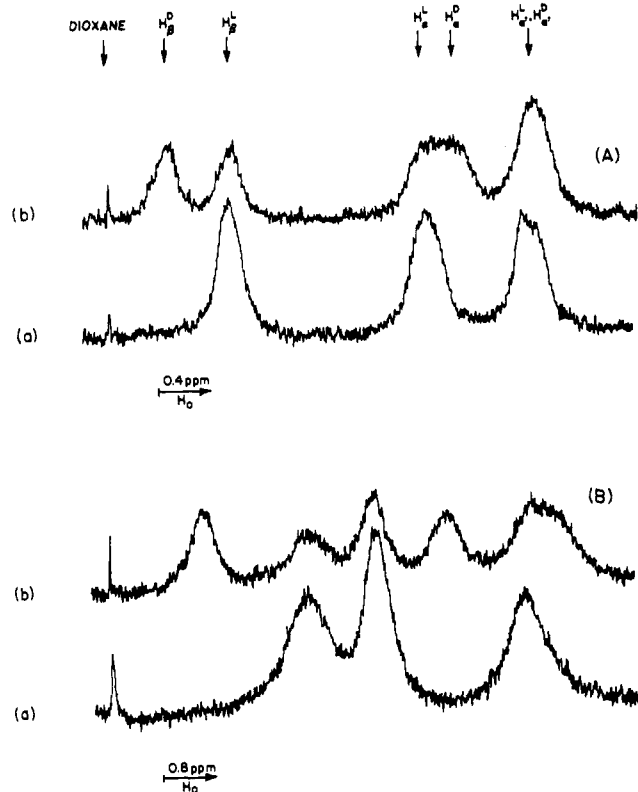
$$1/T_1 = Cr^{-6}f(\tau_c)$$

Figure 2. Proton magnetic resonance spectra at 90 MHz of aqueous (D_2O) solutions at pD 6.4 containing 0.1 M CoATP and (a) 0.17 M L-norepinephrine or (b) 0.17 M racemic norepinephrine at 27 °C (A) and 0 °C (B). The sharp peak on the left is due to a trace of p-dioxane used as an internal reference.

$1/T_1$, being the longitudinal relaxation time.[5] The corresponding distance in the complex of D-norepinephrine is 5.4 Å as calculated using a similar relationship for the transverse relaxation rate.[7] The distances measured on molecular models based on the structures given in Figure 1 are 5.2 and 6.0 Å for the L and D enantiomers, respectively. Bearing in mind that distances derived from NMR data are averages taken over intramolecular rotations that are rapid on the NMR time scale, the agreement between the two sets of distances should be regarded as good. Thus we have good evidence for the preferred conformations of the ethanolamine side chains of L- and D-norepinephrine in their complexes with ATP as depicted in Figure 1.

The results presented here demonstrate that CoATP can be used to resolve NMR spectra of racemic mixtures in aqueous solution; i.e., it can serve as an aqueous chiral shift reagent. In this way the different disposition of the β-hydroxyl group (and the β proton) in L- and D-norepinephrine in their complexes with CoATP has been established. In the complex with ATP or with its metal ion chelate all of the functional groups of L-norepinephrine (the α-ammonium, the β-hydroxyl, and the catechol hydroxyls) are positioned on the same side (cf. Figure 1). This arrangement is impaired for D-norepinephrine in which the β-hydroxyl faces the opposite side. It has been suggested that for maximum physiological effect the attachment to the receptor site must involve three groups: the α-amino, the β-hydroxyl, and the aromatic moiety.[8] Our findings may form a conformational basis for rationalization of the different biological activities of the norepinephrine enantiomers provided that a ternary norepinephrine–ATP–receptor complex is formed.[9]

References and Notes

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(6) Note that upon chelation of a metal the α- and β-phosphorus atoms of ATP become asymmetric resulting in a racemic mixture of enantiomeric metal–ATP complexes.

(7) The relationship between line width (Δ) and transverse relaxation rate ($1/T_2$) is $\Delta = 1/\pi T_2$.

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Norepinephrine Complexes and Reduces Vanadium(V) to Reverse Vanadate Inhibition of the (Na,K)-ATPase

Sir:

Recently, it has been shown that vanadium, which is essential to mammalian life, may be present in muscle tissue at concentrations sufficient to inhibit the sodium and potassium stimulated adenosine triphosphatase ((Na,K)-ATPase).[1] There are several reports of catecholamine activation of (Na,K)-ATPase in vitro,[2] and it has been proposed that these compounds simply reverse vanadate inhibition.[2d] In this communication we present evidence showing that the reversal (and several other related effects) can be explained by a series of complexation and redox reactions between V(V) and norepinephrine (NE).

Catechol and its derivatives react rapidly with VO_2^+ in high acid[3] and with vanadate at physiological pH (i.e., pH 6–8).[4] With excess vanadate, the blue color characteristic of VO^{2+} appears instantaneously upon mixing. At low [V(V)] to [catechol] ratios a yellow color is observed, which upon standing turns brown. The blue color of solutions with initial excess vanadate gradually disappears, resulting in the same brown solution. Both processes require a few minutes, depending on concentration and the catechol derivative; the blue color remains in solutions kept under argon.

The initially observed reaction has been interpreted as a formal redox reaction: $2V(V) + \text{catechol} = 2V(IV) + o\text{-quinone} + 2H^+$.[3,4] The subsequent disappearance of the blue V(IV) produced in the reaction is most likely due to reoxidation by atmospheric oxygen to V(V), a rapid process.[5] The slower conversion of the yellow color to brown can be explained by the well-known polymerization of the organic quinonoid product.[6] This interpretation can be applied to the interaction between vanadate and norepinephrine, a catechol derivative, explaining the reversal of (Na,K)-ATPase inhibition by vanadate.

Spectral studies show that upon mixing vanadate with NE, two new peaks form rapidly: one at 680 nm assigned to the coordinated vanadyl (VO^{2+}) center and one at 295 nm assigned to coordinated ligand. At longer times there is a simultaneous appearance of peaks at 300 and 485 nm (probably a hydroxyquinone). The rate of appearance of the latter peaks may be impeded with dithiothreitol. The addition of MnO_4^-

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