Studies of Molecular Association in Biological Systems by Positron Annihilation Techniques[1]

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Abstract: The positron annihilation technique was applied to determine the complex formation constants for a series of molecular complexes of vitamin K_1 and α-tocopherolquinone with donors such as mono-, di-, and tri-*n*-butylamines, indole, and vitamin D_3 in cyclohexane and benzene solution. The formation constants, K_c, obtained for vitamin K_1 complexes with mono-, di-, and tri-*n*-butylamines and vitamine D_3 all in cyclohexane solution are 0.72, 0.54, 0.24, and 0.83 M^{-1}, respectively. K_c for vitamin K_1 and α-tocopherolquinone with indole as donor in benzene solution was 0.50 and 0.29 M^{-1}, respectively.

It has been postulated that molecular association and the possible formation of charge transfer complexes play an important role in many biological systems.

The theory of molecular association and complex formation and its application to biological systems have been extensively studied and reviewed by several authors.[2]

Thus, the detection and characterization of the mechanism of molecular association or molecular complex formation has become a most interesting topic in current biochemical research.

In a previous paper[3] we have demonstrated and discussed the positron annihilation technique as a method to determine the molecular association constant. We found this technique to be particularly suitable in systems where the formation constant is very small and where spectroscopic methods are not easily applicable due to overlapping in the absorption of the complex and the noncomplexed acceptor molecule.

The positron annihilation method[4] is based on the fact that positronium, Ps, which is the bound state of an electron and a positron, reacts very rapidly with compounds such as nitrobenzene, tetracyanoethylene, etc., which are known as good electron acceptors.[5-6] These compounds, however, lose most of their reactivity toward Ps, if they are present in the form of a charge transfer complex, e.g., as the nitrobenzene–hexamethylbenzene complex, etc.[3,7-8] Thus, it is possible to correlate the observable differences in the reactivity of these molecules in their complexed and noncomplexed form with the molecular association constants of these systems.[3]

In the following we have extended our investigation to the study of molecular association by positron annihilation techniques in biochemical systems. The quinone-related compounds here are of special interest because charge transfer reactions were observed between compounds having quinone structure and biological donor molecules;[9-10] they are widely distributed in nature, and they strongly react with Ps, forming Ps molecule complexes.

Thus in the present work, we chose quinone-related compounds, such as vitamin K_1 and α-tocopherolquinone, as acceptors and studied their interactions with donors, such as indole, vitamin D_3, *n*-butylamine, dibutylamine, and tributylamine in cyclohexane and benzene solution. To the best of our knowledge the molecular association constants have not been reported previously and it appears difficult to assess the formation constants by conventional spectroscopic methods because of the small wavelength shift found in the absorption spectra between neat acceptor and complex.

Experimental Section

A. General Outline of Method Used to Determine Molecular Association Constants via the Positron Annihilation Technique. In the previous paper[3] we have shown that in a solution containing an acceptor molecule, which reacts strongly with Ps, and a donor, which is fairly unreactive toward Ps, the slope, λ_2, of the long-lived component in the positron lifetime spectra is given by the following equation:

$$\lambda_2 = \lambda_P + K_{Q(obsd)}([Q] - [QD]) + K_{QD(obsd)}[QD] \quad (1)$$

where λ_P is the solvent annihilation rate, [Q] is the initial concentration of the acceptor, [QD] is the equilibrium concentration of the complex, and $K_{Q(obsd)}$ and $K_{QD(obsd)}$ are the apparent Ps quenching rate constants for the acceptor and the complex, respectively. They exist in a chemical equilibrium as

$$Q + D \rightleftharpoons QD \quad (2)$$

For a large excess of [D] present, one can approximate [QD] as:

$$[QD] = [Q][D]K_c/(1 + [D]K_c) \quad (3)$$

where K_c is the equilibrium constant for the reaction described in eq 2.

In the previous work, λ_P was considered a constant at different concentrations of [D]. However λ_P can actually be obtained at different concentrations of [D] and the difference between λ_2 and λ_P at any chosen concentration of [D] can be expressed as:

$$\lambda_2 = \lambda_2 - \lambda_P = K_{Q(obsd)}([Q] - [QD]) + K_{QD(obsd)}[QD] \quad (4)$$

One can further obtain the difference between λ_2 and λ_P for the solution of an acceptor in the solvent without donor present as:

$$\lambda_2^0 = \lambda_2^0 - \lambda_P = K_{Q(obsd)}[Q] \quad (5)$$

By proper arrangement of eq 3, 4, and 5, an equation for determining the complex formation constant K_c can be derived:

$$\frac{1}{\lambda_2 - \lambda_2^0} = \frac{1}{K_c[Q][D][K_{QD(obsd)} - K_{Q(obsd)}]} + \frac{1}{[Q][K_{QD(obsd)} - K_{Q(obsd)}]} \quad (6)$$

This method to determine K_c is identical with the one described in the previous work[3] except that here $1/(\lambda_2 - \lambda_2^0)$ is plotted vs. $1/[D]$ instead of $1/(\lambda_2 - \lambda_2^0)$ vs. $1/[D]$ in order to extrapolate $1/[D] \rightarrow 0$ for intercept and slope. The complex formation constant is then obtained by dividing the intercept by the slope. Equation 5 is used to correct the possible change in λ_p at different concentrations of [D].

B. Positron Lifetime Measurements. Positron lifetime measurements were carried out by the usual delayed coincidence method.[11] The resolution of the system as measured by the prompt time distribution of ^{60}Co source and without changing the 1.27 and 0.511 MeV bias was found to be 0.39 ns fwhm. Corrections for the source component, which had an intensity of less than 4%, were made in the usual way by using conventional computational methods.

C. Purity of Reagents. All solvents were of highest available purity and dried by means of a molecular sieve and redistilled. The other compounds used in these investigations were purified by suitable methods, distillation, recrystallization, and preparative gas chromatography, until subsequent test showed a purity of better than 99.5%. Vitamin K_1 and α-tocopherolquinone were obtained from ICN Life Sciences, Inc.

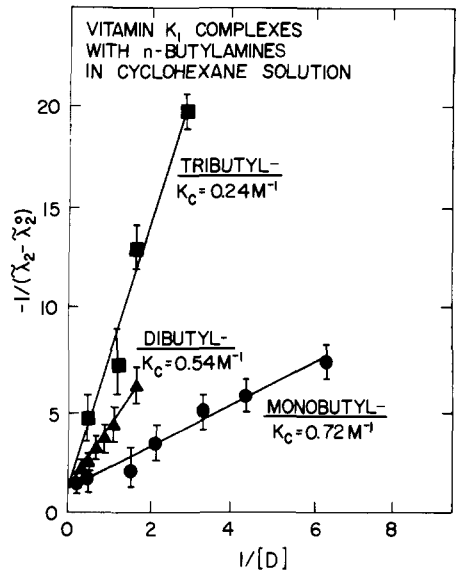

Figure 1. $-1/(\lambda_2 - \lambda_2^0)$ vs. $1/[D]$ for various vitamin K_1-butylamine complexes in cyclohexane solutions. Concentration of vitamin K_1, 32.05 mM; concentration of donor [D] in mol/L; $-1/(\lambda_2 - \lambda_2^0)$ in 10^{-9} s.

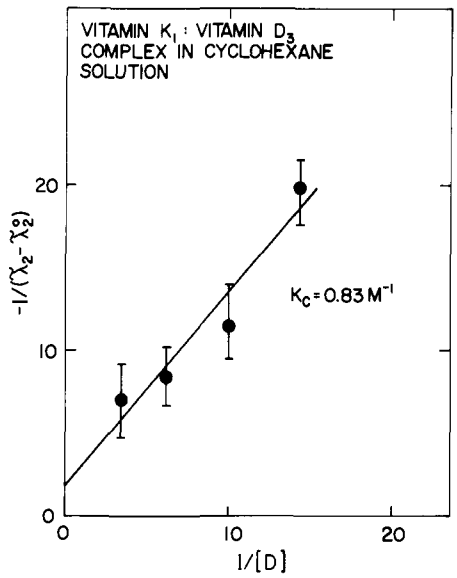

Figure 2. $-1/(\lambda_2 - \lambda_2^0)$ vs. $1/[D]$ for vitamin K_1-vitamin D_3 complexes in cyclohexane solutions. Concentration of vitamin K_1, 26.38 mM; concentration of donor [D] in mol/L; $-1/(\lambda_2 - \lambda_2^0)$ in 10^{-9} s.

D. Preparation of Sample. Specially designed sample vials (cylindrical glass tubes 100 mm long and 10 mm i.d.) were filled with about 1 ml of solution. The positron sources were 3-5 μCi of ^{22}Na prepared by evaporating carrier free neutral solutions of either $^{22}NaHCO_3$ or $^{22}NaCl$ (obtained from Amersham/Searle Co.) onto a thin aluminum foil. The radioactive foils were suspended in the solutions and all solutions were carefully degassed by freeze-thaw techniques to remove oxygen. The vials were subsequently sealed off and the measurements carried out at room temperature. For vitamin K_1-amine complex samples, the vials were stored in the ice bath for more than 48 h, so that the sample solutions reached equilibrium before the start of the lifetime measurements.

Results and Discussion

As it can be seen from Figures 1 and 2, the plot of $-1/(\lambda_2 - \lambda_2^0)$ as a function of the reciprocal donor concentration for vitamin K_1 in cyclohexane solution containing donor molecules such as mono-, di-, and tributylamine and vitamin D_3, results in straight lines as expected from eq 6.

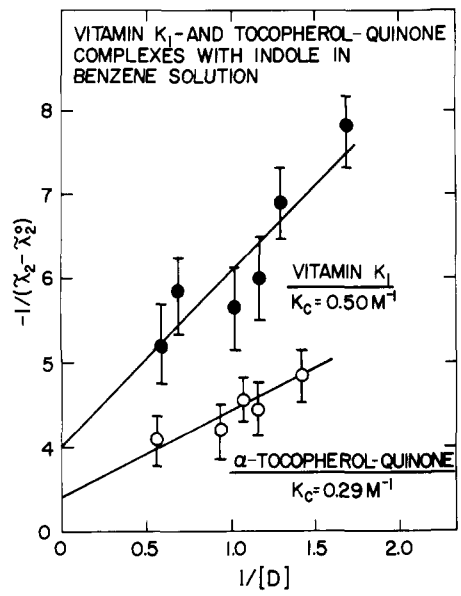

Figure 3. $-1/(\lambda_2 - \lambda_2^0)$ vs. $1/[D]$ for vitamin K_1 and α-tocopherolquinone complexes with indole in benzene solution. Concentration of vitamin K_1, 26.38 mM; concentration of α-tocopherolquinone, 24.54 mM; concentration of donor [D] in mol/L; $-1/(\lambda_2 - \lambda_2^0)$ in 10^{-9} s.

Table I. Complex Formation Constants K_c, the Apparent Rate Constants K_{obsd} for the Acceptors Vitamin K_1 and α-Tocopherol-Quinone and Their Various Molecular Complexes Determined by the Positron Annihilation Technique

Compd	$K_{obsd} \times 10^{10}$ M^{-1} s^{-1}	K_c M^{-1}
Vitamin K_1 in benzene	2.45	
Vitamin K_1-indole in benzene	1.51	0.50
Vitamin K_1 in cyclohexane	1.96	
Vitamin K_1-*n*-butylamine in cyclohexane	Not detectable[a]	0.72
Vitamin K_1-dibutylamine in cyclohexane	Not detectable[a]	0.54
Vitamin K_1-tributylamine in cyclohexane	Not detectable[a]	0.24
Vitamin K_1-vitamin D_3 in cyclohexane	Not detectable[a]	0.83
α-Tocopherolquinone in benzene	2.15	
α-Tocopherolquinone-indole in benzene	0.85	0.29

[a] The reactivity of the molecular complex toward Ps is below the detectable limit ($K_{QD(obsd)} < 0.2 \times 10^{10}$ M^{-1} s^{-1}) inherent to the method of evaluation.

From the intercept and slope of these plots the molecular association constants of these systems as well as the rate constant for the Ps reactions with the complex can be evaluated (vide supra). As pointed out in the Experimental Section, essential to this evaluation procedure is that $K_{QD(obsd)}$ and $K_{Q(obsd)}$ remain practically constant over the whole concentration range studied, which is borne out in the present investigation by the observed linearity of $1/(\lambda_2 - \lambda_2^0)$ vs. $1/[D]$.

The molecular association constants for vitamin K_1 and α-tocopherolquinone complexes with indole in benzene solution were determined in a similar fashion as shown in Figure 3.

The molecular association constants of the systems studied in this investigation are generally very small, indicating that only very weak complexes are formed. It is interesting that K_c observed for the vitamin K_1 complexes with mono-, di-, and tributylamine decreases in this order. The amines and indole

were chosen because these groups are essential components of proteins which are known to act as donors in molecular complex formation in biological reactions with vitamin K_1, which, e.g., are responsible for the maintenance of the normal blood coagulation.[9]

Since it is believed that the charge transfer in these complexes is accomplished by the unpaired electron of the nitrogen to the quinone group of the vitamin K_1 (or α-tocopherolquinone), this effect should be enhanced by the presence of additional *n*-butyl groups. The fact that the opposite was observed, namely that K_c decreases with the number of *n*-butyl groups present, is therefore not the result of an inductive effect, but could be explained in terms of a steric hindrance which makes the formation of the molecular complexes with the higher *n*-butyl substituted donors more difficult.

Vitamin D_3 has been known as a good electron donor in biological molecular complex formation.[12] The results of the present study where the formation constants K_c for vitamin K_1–vitamin D_3 complexes have been assessed in cyclohexane solution (see Figure 2 and Table I) confirm this observation and suggest very similar donor capabilities for vitamin D_3 and mono-*n*-butylamine.

The strong reactivity displayed by α-tocopherolquinone, which is believed to be one of the major products in the oxidation process of vitamin E, toward Ps may open up an interesting possibility of studying the oxidation process of vitamin E.

While α-tocopherolquinone reacts very rapidly with Ps, the rate constant is 2.15×10^{10} M^{-1} s^{-1} in benzene, and is even relatively reactive in its complex with indole ($K_{obsd} = 0.85 \times 10^{10}$ M^{-1} s^{-1}), the nonoxidized vitamin E shows (in benzene solution) hardly any reactivity toward Ps. Thus this drastic difference in the behavior of the oxidation product of vitamin E and vitamin E itself could be utilized for further studies of the biologically interesting autoxidation process of vitamin E, which supposedly inhibits the autoxidation of the unsaturated fatty acids.[2]

Summarizing, it can be said that these initial investigations of molecular associations in biological systems by positron annihilation techniques seem to support the feasibility of this new technique in the study of biological reactions.

References and Notes

(1) Work partially supported by the U.S. Energy Research and Development Administration.
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Regiospecific and Enantioselective Horse Liver Alcohol Dehydrogenase Catalyzed Oxidations of Some Hydroxycyclopentanes[1a,b]

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Abstract: Horse liver alcohol dehydrogenase (HLADH) has been shown to have the ability to retain its enantioselectivity while effecting regiospecific oxidation of only one of two unhindered hydroxyl groups within the same molecule. This provides a synthetically useful combination of properties which cannot be duplicated in a single step by traditional oxidation methods. All reactions were performed on a preparative (up to 1 g) scale and proceeded in good yields. HLADH-catalyzed oxidation of (±)-*cis*-2-(2′-hydroxyethyl)-3-cyclopenten-1-ol (**1**) was regiospecific for the primary alcohol group. The reaction was also enantioselective, and 1*R*,2*S*-*cis*-2-carboxymethyl-3-cyclopenten-1-ol lactone (**4**, 49% optical purity) and unchanged 1*S*,2*R*-**1** (23% optical purity) were isolated. The same regiospecificity, but no enantioselectivity, was observed with (±)-*cis*-2-(2′-hydroxyethyl)cyclopentanol (**2**) as the substrate. Both enantiomers of the prostaglandin synthon **4** were subsequently obtained (37 and 47% optical purities) via HLADH-catalyzed oxidation of the racemic hemiacetal precursor of **4**. For the 1,3 isomer of **2**, (±)-*cis*-3-(2′-hydroxyethyl)cyclopentanol (**3**), secondary alcohol regiospecificity is manifest with 3-(2′-hydroxyethyl) cyclopentanone (**9**) being the major product. The enantioselectivity of this reaction is very high, giving 3*S*-**9** and recovered 1*R*,3*R*-**3** of 97 and 70% optical purities, respectively. The regiospecificities observed were as predicted by the diamond lattice section of the active site. The model is more equivocal, but still useful, in analyzing the enantioselectivities of the above reactions.

In recent years, the requirements of synthetic chemists for reagents capable of effecting selective or asymmetric transformations have increased dramatically. Enzymes present unique opportunities in this regard, and the exploration of their properties as chiral catalysts is now receiving considerable attention.[2] One of the great synthetic attractions of an enzyme