aspartic acid without noticeably accumulating free or bound β-CNAla may be of this type.

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Superoxocobalamin, the First Intermediate in the Autoxidation of Vitamin B_{12r}

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Abstract: Crystalline cob(II)alamin (vitamin B_{12r}) and its solutions in various liquids react reversibly with O_2 to yield a complex which is shown by epr spectroscopy to be mononuclear. In accord with the known shielding of the cobalt atom of the cobalamin against collision with large molecules, the mononuclear product of oxygenation does not combine with a second cob(II)alamin molecule to give a dicobalt complex. Eight hyperfine lines are found in the spectrum of methanolic solutions of oxygenated cob(II)alamin at temperatures close to the melting point. The coupling constant due to the cobalt nucleus is 12 G. At about 159°K there is a transition attributed to cessation of tumbling to give a spectrum different in over-all shape and in having an additional set of eight hyperfine lines. Superhyperfine structure due to the coordinated nitrogen atom of dimethylbenzimidazole is lost on oxygenation. Comparison with the hyperfine coupling constants of related binuclear complexes indicates that oxygenated cob(II)-alamin may be regarded as superoxocobalamin, most of the unpaired spin density being concentrated at the coordinated $O_2 \cdot^-$ group. Detection of a reversibly formed mononuclear product of oxygenation establishes the first reaction step in the autoxidation of B_{12r} and indicates the likelihood of transient occurrence of mononuclear superoxo complexes during autoxidation of hemoproteins. When cob(II)inamides are oxygenated the epr signals obtained resemble those from superoxocobalamin at temperatures below 159°K. The hyperfine coupling constants may be larger or smaller than for superoxocobalamin, depending upon whether the strength of bonding of the fifth ligand is less or greater than that for the nucleotide in the cobalamin.

In many catalyzed autoxidations, both enzymatic and nonbiological, the participation of O_2 begins with its activation, *i.e.*, a weakening of the bond between the two oxygen atoms. Sometimes the activation is effected by an organic free radical (mono or bi), sometimes by a metal atom of an inorganic compound or of a coordination complex. It is the latter class with which we have been principally concerned. Elsewhere we have listed eight conceivable ways in which activation by the metal may commence[1] and have, together with other groups of investigators (see, for example, references 2–4), provided some experimental evidence for the reality of four of them.[1,5-8]

Pauling[9] and many later investigators have studied the reversible complexing of O_2 to give a diamagnetic mononuclear product such as oxymyoglobin. Neither partner behaves as a free radical. The present report is concerned with the combination of O_2 with a free radical-like metal ion to yield a paramagnetic mononuclear product.

$$M\cdot + O_2 \rightarrow MO_2\cdot$$

A complex of this type had previously been detected[6] during the autoxidation of $[Co^{II}(CN)_5]^{3-}$, but we were unable to prove that it could be formed in the initial reaction step, apparently because it reacted swiftly with a second pentacyanocobaltate(II) ion to give a binuclear diamagnetic complex.

$$MO_2\cdot + M\cdot \rightarrow M\text{-}O\text{-}O\text{-}M$$

To avoid the difficulty we have chosen for study the oxygenation of cob(II)alamin (henceforth referred to as B_{12r}), a complex in which the cobaltous ion is shielded against close approach of similarly protected metal atoms.[10]

Some of the later steps in the autoxidation of B_{12r} to aquocobalamin have been elucidated by King, *et al.*[8]

Experimental Section

Materials. Glaxo aquocobalamin was recrystallized from acetone–water before use. Cyanoaquocobinamide was prepared

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(5) J. H. Bayston, R. N. Beale, N. K. King, and M. E. Winfield, *Australian J. Chem.*, **16**, 954 (1963).
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(10) D. C. Hodgkin, quoted by E. L. Smith, L. Mervyn, P. W. Muggleton, A. W. Johnson, and N. Shaw, *Ann. N. Y. Acad. Sci.*, **112**, 565 (1964).

from Glaxo cyanocobalamin by a modification of the procedure of Armitage, *et al.*,[11] and purified by phenol extraction followed by column chromatography, first on carboxymethylcellulose and then (as dicyanocobinamide) repeatedly on DEAE-cellulose. To obtain diaquocobinamide the solution of the cyano complex was desalted, photolyzed[12] in oxygenated HCl solution, pH 2, then chromatographed on carboxymethylcellulose. Elution followed by lyophilization yielded a microcrystalline product which was nearly free of unwanted acid and salt.

Other reagents employed were analytical reagent grade except methanol and N,N-dimethylformamide (both spectroscopic grade) and α-hydroxytoluene (laboratory reagent grade). Buffers used were borate (sodium salts), phosphate (sodium salts), citrate–phosphate (sodium salts), and HCl–KCl, in order of decreasing pH. The final buffer concentration was usually 0.066 *M*.

To obtain the cobaltous corrinoids the cobaltic form was reduced[13] with CO, followed by flushing with argon to remove excess reductant and the CO_2 produced. When the pH was less than 7 or when an organic solvent was used, the reducing agent was potassium formate, excess of which remained throughout the subsequent experimentation. Gas trains for purification of tank argon and carbon monoxide were as described earlier.[13]

Spectrometry. When epr measurements and unpaired spin concentrations were required on solutions to be examined spectrophotometrically (for determination of the initial and final concentrations of aquocobalamin) they were performed as described earlier[6] in a cell in which a quartz epr sample tube was sealed at right angles to a quartz Thunberg cuvette. Less critical epr experiments were carried out as follows: 0.2 ml of aquocobalamin or cobinamide solution, usually 3×10^{-3} *M*, in a 3.5-mm i.d. quartz epr sample tube, 20 cm long, was flushed for 3 min with purified argon, delivered to the bottom of the sample by a 1-mm polythene tube with a very fine tip to minimize bubble size. It was sometimes necessary to cool the upper part of the sample tube with ice to condense solvent vapors. With a second polythene tube the solution was slowly flushed with purified CO for 10 min or more, sufficient time at about 25° to reduce most of the corrinoid to the cobaltous state. After removing the second tube and briefly flushing the solution with argon, the first gassing tube was withdrawn to a point about 5 cm above the solution, the rate of gas flow was considerably increased, and a third polythene tube was inserted to liberate very small bubbles of oxygen at the bottom of the solution, which was held near its freezing point. As rapidly[14] as possible after oxygenation was judged from color change to be on the verge of completion, the sample was frozen in liquid nitrogen while removing the oxygen supply tube. The epr sample tube was then sealed by slowly inserting and rotating a tapered glass rod into a heavily walled tube of butyl rubber, which had been pushed over the end of the sample tube before gassing commenced. Simultaneously the argon supply tube was slowly withdrawn. (Such a closure can prevent entry of air for periods of many weeks, but can be dangerous if the sample tube is allowed to warm to room temperature.) If formate was to be the reductant, a deaerated solution of the formate was anaerobically introduced *via* a gas-tight syringe fitted with a 20-cm needle.

Epr spectra were scanned several times at a rate of 2500 G/25 min. After averaging the results from two to ten experiments, the error in determination of coupling constants was usually about ±0.5 G, while errors in *g* values were about ±0.003 (except in certain experiments in which a sample of Keramot[6] was scanned at the same time as a check on the Fieldial calibration).

If spectra were to be scanned with samples in the liquid state, the solutions were contained either in a Varian "liquid cell" or in a quartz capillary tube, 1.25-mm i.d., sealed to the bottom of a 3.5-mm tube in which the solution was degassed prior to being flipped into the smaller tube.

Results

Oxygenation of Reduced Cobalamin. When O_2 was admitted to an aqueous solution of B_{12r} at 273°K for times of the order of 1 min (the optimum time depending upon pH and concentration), followed by freezing in liquid nitrogen, the characteristic epr spectrum of B_{12r}[13,15,16] was largely replaced by a new signal, with a *g* value of 2.02. The experiment could be carried out with more control by using methanol as solvent and working at 174°K, at which temperature all of the autoxidation steps proceeded slowly (if at all) except the first, which was still rapid. When it was desired to follow the progress of the first step the B_{12r} solution was held in the epr cavity at 159°K, a little air was added to the space above the frozen sample, and the cavity temperature was then allowed to rise slowly until epr absorption began to increase in the vicinity of $g = 2.02$. By repeated scanning, the gradual transformation of B_{12r} to product could be followed until only traces of the reduced complex remained (by increasing amplifier gain to its maximum value *ca.* 0.1% of the cobalamin in the reduced form could be detected). The product signal (after warming to 218°K to enhance the hyperfine structure as in Figure 1A) strongly resembled the eight-line spectrum observed by Bayston, *et al.*,[6] during the autoxidation of $[Co^{II}(CN)_5]^{3-}$ and ascribed by them to the ion $[(CN)_5Co^{III}\text{–}O\text{–}O\cdot]^{3-}$. When the space above the solution of oxygenated B_{12r} was flushed with argon, the epr signal gradually changed back to that of B_{12r} itself. Recovery was high, usually about 90% if the temperature was not allowed to rise much above 174°K.

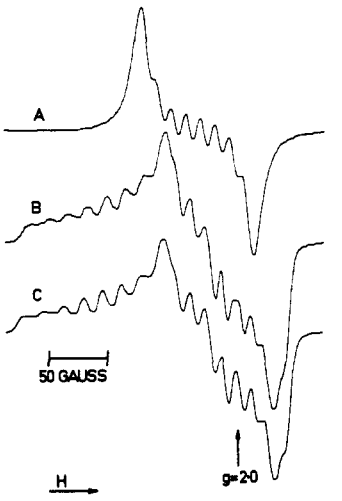


Figure 1. Curve A: epr spectrum of oxygenated B_{12r} in methanol at 218°K (type I signal); complex formed at 174°K; attenuation 20 db; modulation 63. At 174°K the signal is of the same type but is less well resolved. Curve B: spectrum of oxygenated B_{12r} in methanol at 77°K (type II signal); complex formed at 174°K; attenuation 20 db; modulation 63. Curve C: spectrum of oxygenated dimethylbenzimidazolecob(II)inamide in methanol at 77°K; complex formed at 174°K; attenuation 20 db; modulation 63.

A second type of epr signal was found when oxygenated B_{12r} solutions in methanol were examined at the temperature of liquid nitrogen (Figure 1B). In place of the nearly symmetrical signal seen at 174°K were two sets of eight

(11) J. B. Armitage, J. R. Cannon, A. W. Johnson, L. F. J. Parker, E. L. Smith, W. H. Stafford, and A. R. Todd, *J. Chem. Soc.*, 3849 (1953).

(12) G. C. Hayward, H. A. O. Hill, J. M. Pratt, H. J. Vanston, and R. J. P. Williams, *ibid.*, 6485 (1965).

(13) J. H. Bayston and M. E. Winfield, *J. Catal.*, **9**, 217 (1967).

(14) Except when the solvent was water, when it was necessary to freeze the solution in the tip of the sample tube before lowering it slowly into the coolant.

(15) H. P. C. Hogenkamp, H. A. Barker, and H. S. Mason, *Arch. Biochem. Biophys.*, **100**, 353 (1963).

(16) J. A. Hamilton, R. L. Blakley, F. D. Looney, and M. E. Winfield, to be published.

Table I. Characteristics of the Epr Spectra of Oxygenated Cob(II)alamins and Cob(II)inamides Compared with Those[6] of $[(CN)_5Co^{III}O_2\cdot]^{3-}$

Complex[a] oxygenated	Cavity temp, °K	g	Line width, G[b]	Coupling constant, G: Before oxygenation[c]	Coupling constant, G: After oxygenation[d]	Type of spectrum
$[(CN)_5Co^{II}]^{3-}$ in 1 *M* KOH[e]	228	2.007	75	89	9.8	I
Cyanocob(II)inamide	77	2.006 2.07	59	84	9.5 11.5	II
B_{12r}	174	2.02	95	110	12	I
	77	2.004 2.07	94	110	13 15	II
Pyridinecob(II)inamide	77	2.002 2.07	92	113	13 16	II
DMB[f]-cob(II)inamide	77	2.003 2.07	93	113	13 16.5	II
Isoquinolinecob(II)inamide	77	2.003 2.07	92	114	13 16.5	II
Thiocyanatocob(II)inamide	77	2.005 2.08	99	129	15 18	II
Methanolcob(II)inamide	77	2.000 2.07	117	144	16 19	II
Triphenylphosphinecob(II)inamide	77	2.000 2.07	117	92, 95	18 21	II
B_{12r} in AHT[g]	163	2.002 2.07	90	110	13 15	II
B_{12r} in DMF[h]	77	2.002 2.07	92	110	13 16	II
Adeninecob(II)inamide in DMF	77	2.002 2.08	100	117	14 18	II
Triphenylphosphinecob(II)inamide in DMF	77	2.004 2.08	104	130	16 20.5	II
DMF-cobinamide in DMF	77	2.002 2.08	104	140	16 21	II
B_{12r} in 0.1 *M* KOH[e]	77	2.006	91	110	14	II[i]
B_{12r}, pH[e] 8.2	77	2.005	94	110	17	II[i]
B_{12r}, pH[e] 6.0	77	2.004	94	110	17	II[i]
B_{12r}, pH[e] 4.4	77	2.003	99	~110	17	II[i]
B_{12r}, crystalline[j]	169	2.02	95	110		II[k]

[a] The solvent is methanol except where otherwise indicated. [b] The line width (peak to peak) of the derivative signal when the hyperfine structure is ignored. Where two values are given the second refers to the distance from the peak of the first hyperfine line of the low-field region to the highest observed point of the over-all signal. [c] The coupling constant, due to the cobalt nucleus, found at the high-field end of the signal of the cobaltous complex. It is the mean of the distances between the eight principal hyperfine lines. [d] The coupling constant, due to the cobalt nucleus, after oxygenation. Where two values are given the second refers to the eight principal hyperfine lines seen at the low-field end of type II signals. [e] Aqueous solutions (no methanol). [f] 5,6-Dimethylbenzimidazole. [g] α-Hydroxytoluene. [h] N,N-Dimethylformamide. [i] No low-field hyperfine structure resolved. [j] No solvent. [k] No hyperfine structure resolved at high or low field.

hyperfine lines, of which those at higher field ($g_\perp$) corresponded to a coupling constant about the same as in the type I signal while those at low field ($g_\parallel$) corresponded to a somewhat larger coupling constant and mean g value (Table I).

In order to follow the transition from type I signal to type II, a solution of oxygenated B_{12r} in methanol at 174°K was slowly cooled in the epr cavity while scanning continued. The hyperfine structure of the signal gradually weakened until at 159°K the signal appeared to be a compromise between type I and type II, but with no hyperfine lines resolved. During cooling the dielectric constant of the sample decreased considerably until leveling out at 159°K, thus indicating completion of the freezing process. (The freezing point of pure methanol is 174.6°K; the cavity temperatures quoted in this paper were measured with a calibrated thermocouple and were within 2° of the true sample temperature.)

If the sample was warmed to 174°K the type I signal was again observed, but it could not be regained after slow cooling to 153°K; at all temperatures from 174 to 77°K the type II signal was then seen. However the $g_\parallel$ hyperfine structure was well resolved only at very low temperatures. In many samples it was completely lacking at 174°K. As with type I, type II signals gave way to the spectrum of B_{12r} when the samples were flushed with argon at temperatures just below the melting point.

Under no conditions did we detect in spectra due to oxygenated B_{12r} the superhyperfine structure (ascribed to the coordinated nitrogen atom of the nucleotide) which was such a characteristic feature of B_{12r} signals.[16] In acidic solutions of B_{12r} not all of the nucleotide was coordinated to cobalt. As expected, the epr signal of the aquocob(II)alamin which resulted from nucleotide displacement was indistinguishable from that of aquocob(II)inamide.[8] After oxygenation the nucleotide was reattached to the metal atom; only the signal of the oxygenated cob(II)alamin was observed.

When the sample oxygenated was crystalline B_{12r}, the signal obtained was little different from what would be expected for a type II signal in methanol if all hyperfine structure were obliterated. Again the reaction could be

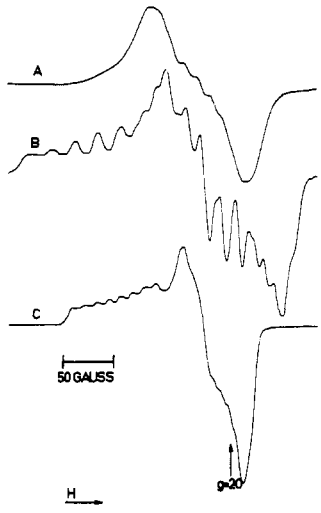


Figure 2. Curve A: epr spectrum of oxygenated isoquinolinecob(II)inamide in methanol at 190°K (mixture of type I and type II signals); complex formed at 174°K; attenuation 10 db; modulation 630. At 77°K the signal is almost identical with that of oxygenated B_{12r} at 77°K. Curve B: spectrum of oxygenated methanolcob(II)inamide in methanol at 77°K; complex formed at 174°K; attenuation 10 db; modulation 630. Curve C: spectrum of oxygenated cyanocob(II)inamide in methanol at 77°K; complex formed at 174°K; attenuation 20 db; modulation 63.

reversed by flushing with argon. Oxygen uptake was observed at temperatures from 163°K upwards. Reaction was not confined to the surface of the crystallites; all of the cobalt atoms were accessible to the gas.

The optical spectra which were recorded during the oxygenation of aqueous solutions of B_{12r} will be described later.[8]

Oxygenation of Reduced Cobinamides. Carbon monoxide proved to be a satisfactory reductant in methanol when the corrinoid to be reduced was diaquocobinamide rather than aquocobalamin. Nevertheless, formate was used as reducing agent in a majority of the experiments performed. To prepare cyanocob(II)inamide it was necessary to reduce before adding the cyanide.[8]

On adding O_2 to aquo- or methanolocob(II)inamide the resulting epr spectra resembled in general features those of oxygenated B_{12r} except that in the type II signals more high-field lines were resolved (Figure 2B), due to less precise overlap of two sets of $g_\perp$ lines.

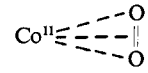
Spectra were also recorded after oxygenation of cob(II)inamides in which the solvent molecule in the fifth coordination position (*i.e.*, approximately perpendicular to the plane of the hydropyrrole nitrogen atoms and on the same side of the plane as the propionamide side chains) had been replaced by dimethylbenzimidazole (Figure 1C), isoquinoline (Figure 2A), cyanide ion (Figure 2C), thiocyanate ion, pyridine, triphenylphosphine, or adenine. The high-field coupling constants proved to be roughly proportional to those[8] of the parent cob(II)inamides, as shown in Table I. In general, oxygenated cobinamides in methanol displayed type I signals only when warmed to 30–40° above the melting point of the solution.

When dimethylbenzimidazole was added to a solution of oxygenated methanolcob(II)inamide in methanol at low temperature, the spectrum of oxygenated dimethylbenzimidazolecob(II)inamide was obtained. Likewise, addition of KCN to a solution of the latter complex resulted in oxygenated cyanocob(II)inamide as the major product, and the only detected paramagnetic product.

Discussion

The presence of 8 hyperfine lines in the type I epr spectrum of oxygenated B_{12r}, together with the failure to detect a 15-line signal under a variety of conditions, confirms the expectation derived from X-ray analysis of the vitamin[10] that oxygenated B_{12r} would prove to be a mononuclear complex. Retention of a hyperfine structure after O_2 addition indicates that the unpaired electron is not far distant from the cobalt atom, but the fall in coupling constant by a factor of about nine suggests that in the new complex the unpaired spin no longer resides principally at the metal atom. The high electronegativity of oxygen leads us to expect that the unpaired electron of B_{12r} has moved close to the oxygen atoms, as in the binuclear peroxo complexes studied earlier.[3,6] We believe that the paramagnetic region of oxygenated B_{12r} is most conveniently represented by the symbolism Co^{III}–O–O· which is one of several canonical forms showing the distribution of the unpaired spin over the three atoms.

The magnitude of the hyperfine coupling constant due to the cobalt nucleus rules out the possibility that the paramagnetic product at an early stage of the autoxidation process is of the type C–O–O·. Other alternatives are



and the analogous structure in which the oxygen molecule is skewed. The resemblances between the epr spectra of the mononuclear oxygen complexes of B_{12r} and $[Co^{II}(CN)_5]^{3-}$ (Table I), along with the demonstration[5,6] that the latter oxygen complex can be derived from $[(CN)_5Co^{III}$–O–O–$Co^{III}(CN)_5]^{6-}$, favor the structure in which one oxygen atom is coordinated to the metal ion.

Weil and Kinnaird[17] have suggested that in some of the paramagnetic binuclear peroxo complexes of cobalt the bridging group is best regarded as a superoxide. Little of the unpaired spin is at the cobalt atoms. Since the isotropic hyperfine coupling constant for oxygenated B_{12r} is close to those of the binuclear oxygenated ammino and cyano complexes of cobalt (12 G compared with ~11 and ~9 G,[5,6,18] respectively), we propose to regard the reversibly formed product of oxygenating B_{12r} as essentially a superoxide ion ($O_2\cdot^-$) coordinated to a formally cobaltic cobalamin. It will be referred to as superoxocobalamin. How much of the unpaired spin is centered on the oxygen atom coordinated to cobalt is a question which must await measurement of the epr spectrum of the ^{17}O analog.

From the greater symmetry of the type I signal and from the conditions under which it is produced it is evident that its form is the result of motional averaging. It can be observed on cooling below the expected freezing point, presumably because, although solvent has frozen out, the solute has been left in droplets of concentrated solution in which tumbling of the paramagnetic ions can still occur. On further cooling, the droplets themselves begin to freeze. In the rigid medium which results the anisotropy

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(18) M. Mori, J. A. Weil, and K. J. Kinnaird, *ibid.*, 71, 103 (1967).

of the paramagnetic species becomes apparent as a more asymmetric epr signal, with separation of $g_{\parallel}$ from $g_{\perp}$, as in type II. To the extent that a crystalline superoxocobalamin phase separates out during freezing of the droplets we expect hyperfine line broadening to occur, with loss of resolution. It is therefore possible that greater resolution than we have attained might be achieved with superoxocobalamin held at a protein surface to prevent close approach of similar ions.

No splitting due to nitrogen nuclei is found after oxygenating B_{12r}, an observation which is in harmony with the concept that the unpaired electron in superoxocobalamin is concentrated in the new ligand. Differences in hyperfine coupling constants for the different superoxo complexes can probably be ascribed to differences in electronegativity of the cobalt atom induced by the fifth ligand. The electron donor properties of cyano ligands (and to a lesser extent the dimethylbenzimidazole ligand in cobalamins) would tend to minimize the odd electron density at the cobalt atom, as pointed out by Mori, *et al.*,[18] for binuclear superoxo complexes.

The finding that all cobalt atoms in microcrystalline B_{12r} are accessible to oxygen at low temperatures suggests that O_2 is able to diffuse through the crystallites along channels such as those described by Brink-Shoemaker, *et al.*,[19] as running continuously throughout wet crystals of cyanocobalamin.

From the ease of oxygenation of methanol solutions of B_{12r} at 174°K it is concluded that the activation energy of the process is almost negligible (less than 1 kcal/mol), as expected for a combination of two free-radical-like species. From a scan at ten times greater gain than that used in recording curve B of Figure 1 it is estimated that the concentration of uncombined B_{12r} is approximately 0.1% of that of superoxocobalamin when equilibrium appears to have been established (total cobalamin concentration 3×10^{-3} *M*). Thus one reason for the stability of superoxocobalamin at low temperatures is the small concentration of B_{12r} available for reduction of the superoxo complex.

The ease with which O_2 combines with B_{12r} at low temperatures suggests direct addition to the cobalt atom rather than substitution for a coordinated solvent molecule. That is, it favors the concept that B_{12r} has only five ligands, as assumed in a previous paper.[13] Elsewhere[8] we shall enlarge upon the ambiguity in this respect of the epr spectra of B_{12r} solutions, which do not permit a clear distinction between solvent effects on the ligands and the effect of weak coordination of a solvent molecule to the cobalt atom.

Epr spectra of B_{12r} produced in methanol or in alkaline aqueous solutions show that after reduction of aquocobalamin the dimethylbenzimidazole group is still coordinated to cobalt,[8] *i.e.*, it still occupies the fifth coordination position. (It is tacitly assumed in the literature on cobalamins that the dimethylbenzimidazole group of the nucleotide side chain cannot coordinate in the sixth position. A Dreiding model of B_{12b} indicates probable steric hindrance to such an attachment. Other evidence regarding differences between the two axial coordination positions has been presented by Müller and Bernhauer.[20]) Oxygen cannot be expected to displace the nucleotide from the fifth position in crystalline B_{12r} at low temperatures, and is therefore assumed to coordinate in the sixth position. If it were in the fifth position, the epr spectrum would be practically the same for oxygenated aquocob(II)inamide as for oxygenated B_{12r}. From similarities in the spectra of superoxocobalamin in the absence and presence of solvents, we conclude that the superoxo group is on the same side of the corrin under all conditions studied.

The order of decreasing coupling constants for cobinamides containing coordinated superoxide (Table I) is approximately the order of increasing strength of binding of the fifth ligand (methanol < adenine[8] < dimethylbenzimidazole < CN^-). The apparent exceptions are the triphenylphosphino complexes; no simple explanation can be given since in the cobaltous state there are two such complexes in methanol and a different one again in dimethylformamide, as indicated in Table I by the several values of the "coupling constant before oxygenation."

Superoxocobalamin is the first fully proven example of a reversibly formed mononuclear product of oxygenation of a cobalt complex. Its reality demonstrates that the superoxide ion, when complexed and particularly when shielded from indiscriminate attack by reducing agents (as it can be, within an enzyme molecule), is sufficiently stable to be a significant intermediate in reactions of hemoproteins with O_2 and H_2O_2. Previously we have assumed this to be so without adequate evidence, for example, in the oxidation of hemoglobin by O_2 in the presence of H donors[21] and in the autoxidation of cytochrome oxidase in the instant before the first peroxo complex is formed.[1]

Recently[22] there has been a proposal that a structure containing the grouping Fe^{II}–O–O·, which is isoelectronic with Co^{III}–O–O·, occurs during reaction of H_2O_2 with cytochrome *c* peroxidase (a ferric complex). The epr spectrum observed at 1.5°K resembles in some respects that of superoxocobalamin.

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(20) O. Müller and K. Bernhauer, *Ann. N. Y. Acad. Sci.*, **112**, 547 (1964).

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(22) J. Peisach, W. E. Blumberg, B. A. Wittenberg, and J. B. Wittenberg, *J. Biol. Chem.*, **243**, 1871 (1968).