The Kinetics of the Aquocobalamin–Thiocyanate Reaction

By DARWIN THUSIUS†

(*Max-Planck-Institut für Physikalische Chemie, Göttingen, Germany*)

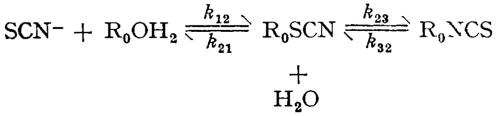
THE kinetics of SCN^- binding to aquocobalamin have been studied by Randall and Alberty by means of the temperature-jump method.[1] A single relaxation time was observed which exhibited a first-order dependence on anion concentration, leading to apparent rate constants for the formation and dissociation of R_0SCN.[2] We have studied the same reaction, but over a wider concentration range, and found the relaxation spectrum to consist of three time-constants. Evidence is presented which suggests that the two additional effects arise from a linkage isomerization of SCN^- and a conformational change in the corrin ring.

Using the *T*-jump method, three relaxations are observed which, at low $[SCN^-]$, are well separated on the time axis. The smallest constant, τ_{III}^{-1}, is concentration dependent and corresponds to the relaxation observed by Randall and Alberty.[1] Above *ca.* 0·01M-$[SCN^-]$ the effect becomes negligible due to complete formation of R_0SCN. At 25° and $\mu = 0{\cdot}5$M, the formation and dissociation rate constants determined from a plot of τ_{III}^{-1} *vs.* ($[SCN^-] + [R_0OH_2]$) are $1{\cdot}9 \times 10^3$M^{-1} sec.$^{-1}$ and $2{\cdot}1$ sec.$^{-1}$, respectively. On the other hand, τ_{II}^{-1} is concentration-independent and equal to 40 sec.$^{-1}$. The fastest relaxation, τ_I^{-1}, cannot be separated from the heating time of the instrument (in this case $\sim 3 \times 10^{-6}$ sec.$^{-1}$) and could not be studied as a function of concentration. The effect may therefore consist of more than one time constant. In contrast to τ_{III}, the amplitudes of both τ_I and τ_{II} increase with increasing $[R_0SCN]$ and reach a maximum when essentially all of the aquo-complex is converted into product. This behaviour is good evidence that τ_I and τ_{II} are due to intramolecular transformations in R_0SCN. The conditions of the *T*-jump experiments were: [cobalamin] $= 0{\cdot}7$—3×10^{-4}, $(SCN^-) = 1{\cdot}0 \times 10^{-3}$—0·50M, perchlorate medium, pH 4—5 (no buffer), $\Delta T = 12°$, and $\lambda = 567$ mμ.

The occurrence of three relaxation times indicates the presence of two cobalt species in addition to R_0OH_2 and R_0SCN. Stopped-flow experiments (25·5°, $\mu = 0{\cdot}5$M, $[\text{cobalamin}]_0 = 2$—5×10^{-5}M, pH 4—5, $\lambda = 500$ mμ, dead time $= 3$ msec.) confirm the existence of one of these additional components. Below 0·03M (SCN–) the overall reaction is a one step process; the formation and dissociation rate constants are $2{\cdot}3 \times 10^3$M^{-1}sec.$^{-1}$ and $1{\cdot}8$ sec.$^{-1}$. At higher ligand concentrations, however, the anation takes place in two discrete steps, The data can be interpreted in terms of a ligand-dependent substitution reaction followed by a first order isomerization. The apparent rate constant for the second step is 40 sec.$^{-1}$, which is identical to τ_{II}^{-1}. Since we find the product spectrum to be independent of $[SCN^-]$ under the conditions prevailing in the stopped-flow experiments, it is unlikely that the observed optical density changes are due to a change in stoicheiometry at high ligand concentrations.

Apparently the rapid transition giving rise to τ_I is common to a number of other cobalamins, all exhibiting an "immeasurably fast" time constant.[3] In view of the recent evidence favouring the existence of temperature-dependent conformational changes in cobalamins,[4] the observed relaxations may well arise from a rapid equilibrium between two conformers. The high rates ($k \gg 2 \times 10^5$ sec.$^{-1}$) are consistent with an estimated ΔH of 2—3 kcal. for such a transformation.[4]

τ_{II} Is provisionally assigned to a linkage isomerization. This is consistent with the ligand-independent nature of τ_{II}^{-1}, the stopped-flow results, and the failure to find an analogous reaction with cobalamins where linkage isomerization is not possible (*e.g.*, R_0N_3 and R_0I).[3] Although thiocyanate is known to be bound through the sulphur atom in the crystalline complex,[5] the kinetic data imply that *ca.* 5—10% of the cobalamin exists as R_0NCS in solution. The following minimal scheme rationalizes both the stopped-flow and *T*-jump results and is consistent with the reported stability constant.[6]



$$SCN^- + R_0OH_2 \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \underset{\displaystyle + \atop \displaystyle H_2O}{R_0SCN} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} R_0NCS$$

Here R_0SCN refers to a mixture of two (or more) conformers in rapid equilibrium. The rate constants are: $k_{12} = 2{\cdot}0 \times 10^3$M^{-1} sec.$^{-1}$, $k_{21} = 2$, $k_{23} \sim 4$, and $k_{32} = 36$ sec.$^{-1}$ at 25°.

There has been considerable interest recently in the linkage isomerization of thiocyanato-complexes,[7] but in only a few cases have rate

---

† Present address: Laboratoire D'Enzymologie, Faculté des Sciences, Orsay, France.

constants been reported.[8,9] The novelty of the cobalamin system lies in what appears to be a rather close balance between *N*- and *S*-bonding, which enables both the anation and isomerization reactions to be followed by simply perturbing the equilibrium.

Postdoctoral stipends from the National Institutes of Health and the Max-Planck-Institut are gratefully acknowledged.

(*Received, July 4th,* 1968; *Com.* 893.)

---

[1] W. C. Randall and R. A. Alberty, *Biochem.* 1966, **5**, 3189.

[2] R_0X represents the cobalamin molecule with the cobalt–benzimidazole bond intact.

[3] D. Thusius, unpublished observations.

[4] R. A. Firth, H. A. O. Hill, J. M. Pratt, R. J. P. Williams, and W. R. Jackson, *Biochem.*, 1967, **6**, 2178.

[5] Cited in ref. 6.

[6] J. M. Pratt and R. G. Thorp, *J. Chem. Soc.* (*A*), 1966, 187. According to this scheme, the observed stability constant (1200 M^{-1}) should be equal to $k_{12}/k_{21}\,(1 + k_{23}/k_{32})$.

[7] See, *e.g.*, J. L. Burmeister and F. Basolo, *Inorg. Chem.*, 1964, **3**, 1587; J. L. Burmeister, *ibid.*, 1964, **3**, 919.

[8] A. Haim and N. Sutin, *J. Amer. Chem. Soc.*, 1965, **87**, 4210; *ibid.*, 1966, **88**, 434.

[9] J. L. Burmeister and F. Basolo, *Inorg. Chem.*, 1964, **3**, 1202; F. Basolo, W. H. Baddley, and K. J. Weidenbaum, *J. Amer. Chem. Soc.*, 1966, **88**, 1576.