Mechanism of Coenzyme-B_{12}-dependent Molecular Rearrangements. Evidence for a Radical-like Process in a Model Reaction for Methylmalonyl–CoA Mutase

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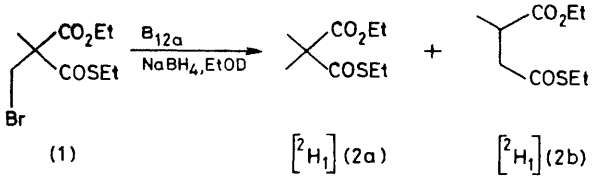
Summary Mechanistic probes of a model system for the coenzyme-B_{12}-dependent methylmalonyl–CoA mutase reaction have suggested (i) that the C–Co bond in the alkylcobalamin intermediate is capable of both carbanionic and homolytic cleavage, and (ii) that the skeletal rearrangement most probably takes place at the radical stage.

THE mechanisms of coenzyme-B_{12}-dependent molecular rearrangements continue to provide both experimental and theoretical challenge.[1–3] Recently, we have reported that an overall anionic mechanistic feature was probably present in a model system for the coenzyme-B_{12}-dependent methylmalonyl–CoA mutase reaction, and it was pointed out that carbanionic deuteriation *per se* (Scheme 1) does not necessarily support the notion that the rearrangement step occurs at the stage of a carbanion intermediate.[4] In this communication, we describe some experiments which bear on this question.

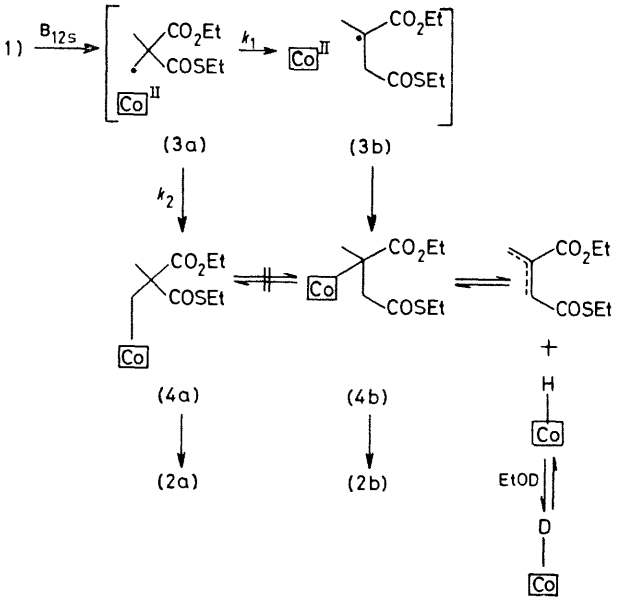


SCHEME 1

Close examination of the model system raises the possibility that the observed carbanionic character might be a reflection of the C–Co bond cleavage process of the alkylcobalamin intermediates [(**4a**) and (**4b**) in Scheme 2] in the presence of a large excess of $NaBH_4$ normally present in the reaction mixture† [equation (1)]. Indeed, the excess of

$$B_{12a} \xrightarrow{i} [B_{12s}] \xrightarrow{ii,\ iii,\ iv} \text{products (\textbf{2a}) and (\textbf{2b})} \quad (1)$$

i, $NaBH_4$, EtOD, ii, RBr, iii, MeCHO, iv, Decomp., see the Table.



SCHEME 2

$NaBH_4$ used in the preparation of the alkylcobalamin intermediates can be effectively destroyed by addition of acetone or acetaldehyde following the formation of B_{12s} or the C–Co bond. We have repeated the thio ester model reaction under the latter conditions to produce the two reported products [(**2a**) and (**2b**)] together with some starting material. The deuterium contents of the products (determined by g.c.–mass spectral analysis) are summarized in the Table.

The data reveal two significant features. Firstly, the amount of deuterium incorporated into the product (**2a**) from EtOD is considerably lower in these runs than in the identical experiment in the presence of an excess of $NaBH_4$.‡

TABLE

Conditions (iv)[a]	(**2a**) ($^2H_0/^2H_1/^2H_2$)[b]	(**2b**) ($^2H_0/^2H_1/^2H_2/^2H_3/^2H_4$)[b]
—[c]	100/8·4/1·1	100/7·9/1·0/0/0
Decomp., dark, room temp., N_2	55/100/10·8	13·3/84·1/100/26·8/2·2
Decomp., dark, 60 °C, N_2	98·9/100/9·4	17·4/79·0/100/38·3/3·1
Decomp. dark, room temp., O_2	47·5/100/10·2	20·6/100/11·5/1·2/0·1

[a] *Cf.* equation 1. [b] Relative abundance based on the major fragment of *m/e* 143 (M^+ — SEt) determined at 15 eV on a Hewlett-Packard g.c.-mass data system 5982-A. [c] Authentic unlabelled samples.

† A typical model experiment is run as follows: Hydroxocobalamin (B_{12a}) in H_2O (or ethanol) is reduced with a large excess of $NaBH_4$ under N_2 (or Ar) atmosphere to B_{12s}. To this mixture in the dark was added the substrate [*e.g.*, (**1**)] in ethanol. The C–Co bond formation is readily observed by u.v. spectroscopy. The mixture was then allowed to decompose in the dark at room temperature.

‡ The previously observed deuterium incorporation under the usual reaction conditions was almost quantitative (ref. 4).

Since the percentage of deuterium in the product is a measure of carbanionic character, it is now clear that presence of an excess of $NaBH_4$ in the reaction mixture enhances the carbanionic cleavage of the C–Co bond and, furthermore, the presence of both [$^2H_0$](**2a**) and [$^2H_1$](**2a**) suggests that the C–Co bond in the alkylcobalamin intermediate is capable of both carbanionic and homolytic cleavage. Secondly, the multiple deuterium incorporation (up to $^2H_3$ or $^2H_4$) and the relative distribution pattern in the rearranged product (**2b**) are features reminiscent of those observed in the reversible addition of a cobalt hydride species to olefins.[5] We believe that the above experiment provides strong evidence for the involvement of the rearranged alkylcobalamin (**4b**), which is capable of undergoing a rapid, reversible β-hydride elimination process. The fact that (**2a**) does not contain more than one deuterium label precludes the conversion of (**4b**) into (**4a**).

Next, we examined the possible rearrangement of the alkylcobalamin (**4a**) to (**4b**). When the bromo substrate (**1**) was treated with B_{12s} at −78 °C in the dark under N_2, the C–Co bond was readily formed. Aliquots were taken (−78 °C to room temp.) at regular intervals, treated with HCl, and the product ratios (**2a**)/(**2b**) determined by g.c. analysis (5 ft column/10% FFAP on Anakrom-60/70-SD).§ The product ratio [(**2a**)/(**2b**) = *ca.* 1/2] was found to remain constant for all the aliquots, suggesting that the ratio of (**4a**) and (**4b**) was already established at the C–Co bond-forming stage. This result, and the observation that a related tosylate¶ failed to react with B_{12s} over a prolonged period, strongly suggest that the initial C–Co bond formation between the bromo substrate (**1**) and the B_{12s} nucleophile may proceed by an electron-transfer mechanism[1d,6] rather than by the S_N2 process as commonly believed,[7] and that the skeletal rearrangement most likely takes place at the radical stage, (**3a**)→(**3b**), rather than (**4a**)→(**4b**). It appears reasonable to assume that the radical rearrangement proceeds at a rate comparable with that of the bimolecular recombination between the alkyl radical (**3a**) and the paramagnetic B_{12r} species[8] (Scheme 2). It remains for us to point out that for a model reaction to be relevant to the mechanistic discussion of a coenzyme-B_{12}-dependent enzymatic reaction, the mechanisms of not only C–Co bond cleavage but also of C–Co bond formation should be clearly understood.

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§ The validity of this assay method is supported by the following experiment. An alkylcobalamin was readily prepared by the reaction of hex-5-enyl bromide with B_{12s} at −78 °C under N_2 in the dark. The slow conversion of hex-5-enylcobalamin to cyclopentylmethylcobalamin at room temperature could be conveniently monitored by this analysis method. Initially only hex-1-ene was observed after HCl quenching, and the ratio of methylcyclopentane to hex-1-ene increased with reaction time, with methylcyclopentane predominating after overnight reaction. This observation also provides evidence for a radical-like mechanism for the alkylcobalamin rearrangement.

¶ The B_{12s} nucleophile readily reacts with diethyl methyl–bromomethylmalonate at room temperature while no appreciable reaction could be observed between B_{12s} and the corresponding tosylate over a 24 h period at that temperature.

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[7] G. N. Schrauzer and E. Deutsch, *J. Am. Chem. Soc.*, 1969, **91**, 3341; F. R. Jensen, V. Madan, and D. H. Buchanan, *ibid.*, 1970, **92**, 1415. It appears likely that ordinary primary and secondary halides and sulphonates react with B_{12s} by an S_N2 mechanism, while the alkyl halides normally inert to S_N2 attack, *e.g.*, neopentyl and cyclopropyl types, might involve the electron-transfer process.

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