SUPPLEMENTARY MATERIAL

Structural basis for adenosylcobalamin activation in AdoCbl-dependent ribonucleotide reductases

Karl-Magnus Larsson[1,4], Derek T. Logan[3,*], and Pär Nordlund[1,2,*]

[1] Dept. of Biochemistry and Biophysics, Stockholm University, S-106 91 Stockholm, Sweden

[2] Dept. of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden

[3] Dept. of Biochemistry and Structural Biology, Lund University, Box 124, S-221 00 Lund, Sweden

[4] Present address: Dept. of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305-5126, USA

*To whom correspondence should be addressed: D.T.L., Dept. of Biochemistry and Structural Biology, Box 124, 221 00 Lund, Sweden, +46 46 222 1443; fax +46 46 222 4692, derek.logan@mbfys.lu.se, or P.N. Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77 Stockholm, Sweden, +46 8 5248 6860, fax + 46 8 5248 6850, par.nordlund@ki.se.

Interpretation of substrate and loop 2 electron density in the dTTP/GDP/AdoCbl complex

Interpretation of the electron density in the substrate area of the dTTP/GDP/AdoCbl complex was complicated by the apparent presence of a significant amount of GDP bound in an unproductive, "backwards" conformation, in which the β-phosphate group of the "backwards" conformation overlaps with the α-phosphate in the true orientation (Fig. S1). A similar phenomenon has been reported for class III RNR, where high concentrations of allosteric effector dNTPs resulted in their binding in a "backwards" conformation to the active site (1). The significance of such conformations is still unknown. However, since the true GDP conformation in this complex is identical to that in all other complexes involving GDP (this work and (2)) we are confident that the observed interaction with the C-propionamide group of AdoCbl is biologically relevant. The best agreement was obtained using a model consisting of 50% true and 50% "backwards" orientation.

The poor occupancy of the true GDP conformation may partly be explained by partial oxidation of the Cys134–Cys333 disulphide bond in this complex, despite the presence of 10 mM DTT in all solutions. It has been shown for class I RNR that disulphide reduction is a prerequisite for substrate binding (3). On the other hand, no large conformational changes in the main chain in the vicinity of the disulphide bond, such as those observed for class I RNR, are seen between the oxidised and reduced forms of tmNrdJ.

Loop 2 bridges the allosteric specificity effector and substrate sites, and is important for communication between them. Loop 2 does not assume an unambiguous conformation in the dTTP/GDP/AdoCbl complex. The best model was obtained by combining the dataset from the 3h soak reported in the main article text with a highly isomorphous dataset from a 24h soak using the same components, in which the electron density for AdoCbl and substrate, was poorer (Supplementary Table 1). A convincing model for loop 2 could be built into the resulting electron density, and when this model was refined against the 3h data set alone, agreement with the electron density was good. However we were unable to model the side chains of residues Arg206 and Arg207. Arg208, which in the dTTP/GDP (2) and dTTP/GDP/Ado complexes stacks across the top of the substrate base to interact with

the β-phosphate group, is oriented away from the substrate. Overall loop 2 has a conformation distinct from that in the two other complexes involving GDP.

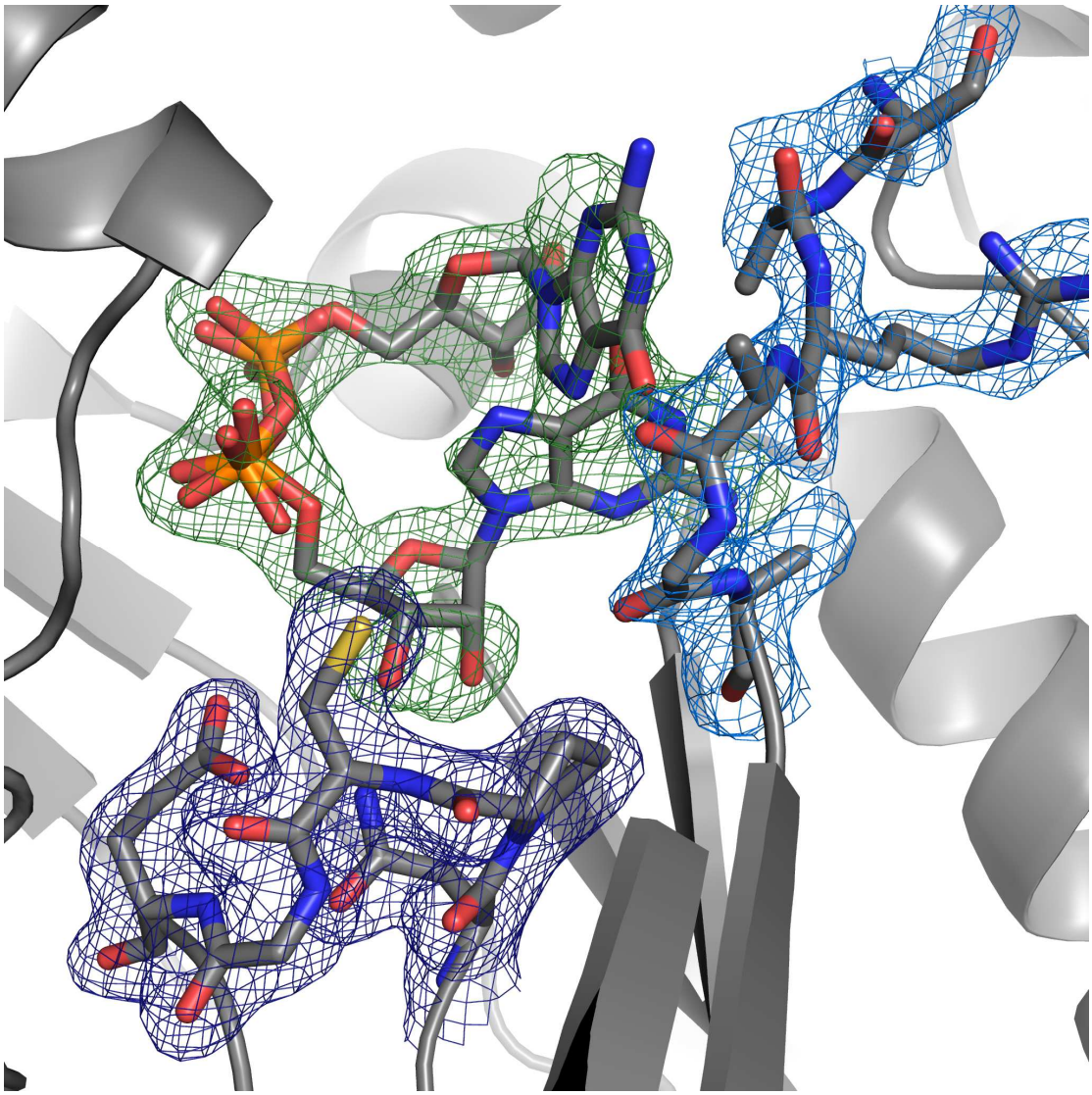
Supplementary Table 1

Details of the combined data set used to build the loop 2 model: A data set from a crystal soaked with dTTP, GDP and AdoCbl for 3 hours was combined with one from a 24h soak.

	dTTP/GDP/ AdoCbl (3h)	**dTTP/GDP/ AdoCbl (24h)**	**dTTP/GDP/ AdoCbl (3h+24h)**
Effector (mM)	0.4	0.4	n/a
Substrate (mM)	2	2	n/a
Cofactor (mM)	~25	~12.5	n/a
$MgCl_2$	8	8	n/a
Beamline	ESRF ID29	MAX-lab I711	n/a
Resolution	49-1.90 (2.00-1.90)	38-1.90 (2.00-1.90)	49-1.90 (2.00-1.90)
Unit cell dimensions			
a,b,c (A)	120, 124, 107	120, 124, 107	120,124,107
β (deg)	102.6	102.7	102.6
Completeness (%)	99.4 (99.6)	99.6 (99.9)	99.6 (99.8)
Observed reflections	530401	499307	1031530
Unique reflections	119789	119197	119977
$\langle I/\sigma \rangle$	12.09 (1.60)	7.88 (1.43)	10.53 (1.92)
R_{merge}	6.4 (84.8)	9.6 (93.8)	12.9 (99.3)

Supplementary Figure 1

Interpretation of electron density for substrate and loop 2 in the dTTP/GDP/AdoCbl complex: A 2m|F_o|-D|F_c| electron density map is shown, calculated with phases from a model containing 50% GDP modelled in the expected conformation and 50% in an unexpected "backwards" conformation. The electron density map is contoured at 1.0 σ, in green mesh around GDP and in blue mesh around loop 2 and the finger loop. Brackets around the single letter amino acid code indicate that the side chain has not been modelled due to lack of electron density.



Supplementary Movie 1

Animation showing the steric clashes that would result from a pure rotation of the adenosine ribosyl group around the glycosidic bond. The animation starts with C5' in the position seen in the dTTP/GDP/AdoCbl complex, at 3.0 Å from the Co atom. The dihedral angle is -180°. It is then swung in the direction of Cys322. At a dihedral angle of -105° the C5' atom approaches the corrin ring most closely, coming to within 2.0 Å of C11. At its distance of closest approach to Cys322, namely 2.0 Å, it is still within van der Waals' contact distance to C46, a D-ring substituent. Thus pure ribosyl rotation cannot explain radical transfer in class II ribonucleotide reductases, at least not without some relaxation of the corrin ring.

References

1. Larsson KM, Andersson J, Sjoberg BM, Nordlund P, & Logan DT (2001) Structural basis for allosteric substrate specificity regulation in anaerobic ribonucleotide reductases. *Structure* 9(8):739-750.
2. Larsson KM*, et al.* (2004) Structural mechanism of allosteric substrate specificity regulation in a ribonucleotide reductase. *Nat Struct Mol Biol* 11(11):1142-1149.
3. Eriksson M*, et al.* (1997) Binding of allosteric effectors to ribonucleotide reductase protein R1: reduction of active-site cysteines promotes substrate binding. *Structure* 5(8):1077-1092.