TABLE 4. X-ray data collection and refinement statistics of the *R. palustris* RLP2 structure

Parameter[b]	Value
Wavelength (Å)	0.9537
Temp (K)	100
Space group	$P2_12_12_1$
Cell parameter (Å)	
a	68.66
b	119.53
c	203.04
Resolution (Å)	103.1–3.3 (3.4–3.3)
No. of reflections	
Total	163,935
Unique	26,399
Completeness[a] (%)	99.7 (99.5)
$<I/\sigma>$[a]	9.6 (3.8)
R_{sym}[a] (%)	16.4 (46.7)
Model refinement	
R/R_{free}[a] (%)	20.3/23.2 (26.8/30.1)
No. of protein atoms	13,016
RMS bond length (Å)	0.012
RMS bond angles (°)	1.396
Ramachandran plot [no. of residues (%)]	
Most favored	1,236 (88.0)
Additional allowed	147 (10.5)
Generously allowed	17 (1.2)
Disallowed	4 (0.3)

[a] Statistics for the outer resolution shell are given in parentheses.
[b] $R_{sym} = \Sigma(I - <I>)^2/\Sigma I^2$; *R*, *R* factor; R_{free}, subset of reflections not included in structure refinement; *I*, intensity of reflections; RMS, root mean square.

palustris RLP2, residue R327 appears to take up a different conformation compared to that in the *C. tepidum* RLP structure (Fig. 10). In *R. palustris* RLP2, the side chain of R327 adopts a conformation comparable to that of K334, the corresponding catalytic residue of spinach (form I) RubisCO (PDB accession number 8RUC). The *R. palustris* RLP2 structure further supports the hypothesis that residue R327 can potentially form hydrogen bonds with the P1 phosphate and the backbone of a CABP-like ligand. Residue E119, although adopting a different conformation relative to the identical residue in *C. tepidum* RLP, can still potentially form a hydrogen bond with the backbone of a CABP-like ligand.

More recently, structure-function studies of a YkrW-type RLP from *Geobacillus kaustophilus* (previously *Bacillus stearothermophilus*) established the structural basis for the "enolase" function of YkrW. Evidence points to the involvement of Lys-98 in proton abstraction, with this residue likely serving as the general base during catalysis, much as Lys-201 (or its equivalent in different forms) serves as the general base during RubisCO catalysis (Fig. 8) (33). Interestingly, Lys-173 of *B. kaustophilus* RLP, which is structurally analogous to Lys-201, is also carboxylated and coordinates with Mg^{2+} when a substrate analog is bound. The compound 2,3-diketohexane-1-phosphate (DK-H-1-P), a substrate analog of 2,3-diketo-5-methylthiopentyl-1-phosphate, was shown to be bound to *G. kaustophilus* RLP in a manner similar to that of the binding of 2-CABP to RubisCO's active site, providing further credence to the conserved means by which the YkrW "enolase" and RubisCO initiate catalysis albeit with differently positioned Lys residues serving as the general bases. While Lys-98 is highly conserved in the YkrW group of proteins, three of which have now been shown to act as enolases in the methionine salvage pathway (7, 11, 33), it is clear that other RLPs possess different residues in this position, especially asparagine, identical to the conserved Asn-123 in bona fide RubisCOs (Fig. 3). This may be a further indication of different functions for RLP in organisms that lack a methionine salvage pathway. Alternatively, for organisms that do utilize RLP in a presumptive methionine salvage pathway, such as *R. rubrum* and *R. palustris* (Fig. 4), but that do not possess a Lys in this position (Fig. 3), it will be interesting to determine the general bases and their locations. In *Bacillus clausii*, an apparent YkrW-type RLP substitutes an arginine for the Lys in position 98 (Fig. 3), raising the question of whether this protein is active in the enolase reaction or perhaps uses Arg as the base to initiate the reaction or even catalyzes some alternative reaction. Likewise, the Glu in position 119 of the *C. tepidum* protein is intriguing, and, from structural considerations, it was suggested that this protein could utilize some unknown ketose phosphate substrate (33), much like the above-described analyses that indicated that this protein binds a substrate that is similar to yet smaller than RuBP (39). The residue dissimilarities at the P2 binding site in

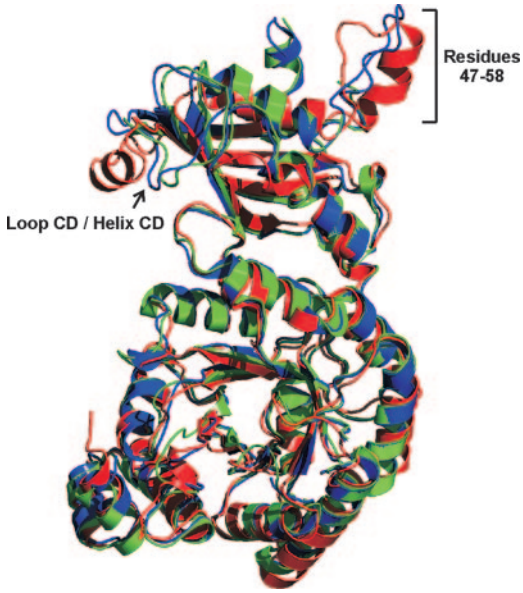

FIG. 11. The monomer structures of RLP2 from *R. palustris* and RLP from *G. kaustophilus* superimposed with the RLP from *C. tepidum*. *R. palustris* RLP2 is blue, *G. kaustophilus* RLP is red, and *C. tepidum* RLP is green. The root mean square deviation (RMSD) of the Cα atom is 0.8 Å between *R. palustris* RLP2 and *C. tepidum* RLP, 1.3 Å between *R. palustris* RLP2 and *G. kaustophilus* RLP, and 1.3 Å between *C. tepidum* RLP2 and *G. kaustophilus* RLP. Two main structural differences can be seen in the N-terminal domain: loop CD in *C. tepidum* RLP and *R. palustris* RLP2 becomes a helix in *G. kaustophilus* RLP, and residues 47 to 58, missed in *C. tepidum* RLP, become a loop in *R. palustris* RLP2 and partly a helix in *G. kaustophilus* RLP.