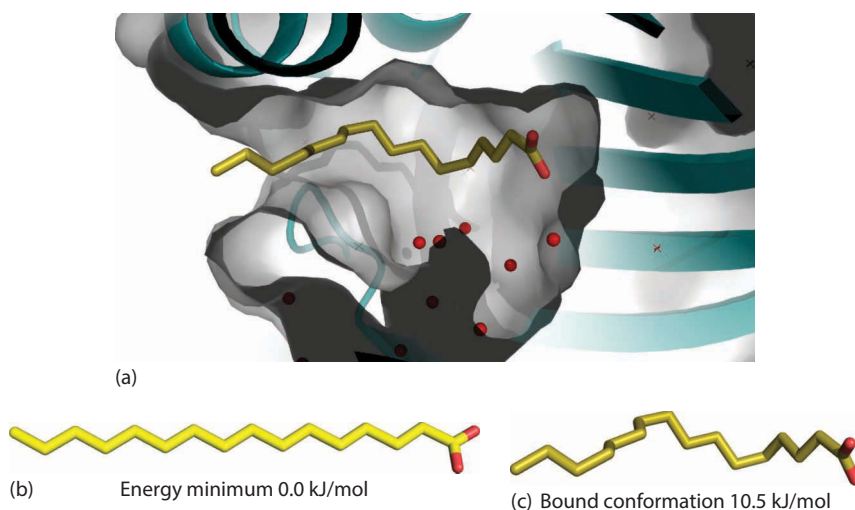


conformations of different ligand shapes. A ligand generally binds to a protein in a single well-defined conformation that positions functional groups used for binding in appropriate locations in space for interactions with their binding partners in the protein. This implies that the motions corresponding to the conformational freedom in aqueous solution are to a large extent frozen in the binding site. As discussed earlier for  $\Delta G_{\text{transl+rot}}$ , this leads to a decrease in the entropy (conformational entropy) giving a more negative  $\Delta S$  and  $T\Delta S$  and thus a free energy cost for binding. The magnitude of  $\Delta G_{\text{conf}}$  due to  $T\Delta S_{\text{conf}}$  has been estimated to be 1–6 kJ/mol per restricted internal rotation and depends on the “tightness” of the ligand–protein complex as in the case of  $\Delta G_{\text{transl+rot}}$  (Section 2.3.1). To minimize the cost associated with freezing of internal motion, one may consider constraining the ligand by introduction of double bonds or by incorporating ring structures.

A second and very important energy contribution to  $\Delta G_{\text{conf}}$  comes from the cost associated with the ligand adopting its bioactive conformation. The ligand conformation in aqueous solution is not necessarily the same as the protein bound conformation and this difference in energy is reflected directly in the binding affinity. Comparisons of ligand conformations observed in X-ray structures of ligand–protein complexes and ligand conformations in aqueous phase (as calculated by state-of-the-art computational methods) show that a ligand in general does not bind to the protein in its preferred conformation (lowest energy conformation) in aqueous solution. An example of this is shown in Figure 2.4. Palmitic acid prefers the well-known all-anti (zigzag) conformation of the hydrocarbon chain in aqueous solution, but binds to the adipocyte lipid-binding protein with an affinity ( $K_i$ ) of 77 nM in a significantly folded conformation. The energy required for palmitic acid to adopt the binding conformation has been calculated to be 10.5 kJ/mol. This conformational energy penalty is detrimental to binding and has the effect of increasing the  $K_i$  value in comparison to a case in which the ligand binds in its preferred conformation in aqueous solution. As shown in Section 2.2, a conformational energy penalty of 5.9 kJ/mol corresponds to a decrease in affinity (increase of  $K_i$ ) by a factor of 10. The 10.5 kJ/mol penalty for palmitic acid thus corresponds to an almost 100-fold loss of affinity. It is consequently of high importance to avoid introducing significant conformational energy penalties when designing new ligands. Calculations of the conformational energy penalties for ligands in a series of X-ray structures of ligand–protein complexes indicate that these energy penalties in general are below 13 kJ/mol. This may be used as a rule of thumb in ligand design. In this context, it is important to note that in calculations of conformational energy penalties, the conformational properties of the unbound ligand “in aqueous phase” must be used as the reference state



**FIGURE 2.4** (a) Palmitic acid bound to the adipocyte lipid-binding protein (pdb-code 1LIE), (b) the preferred conformation of palmitic acid in aqueous solution and (c) the conformation bound to the protein.