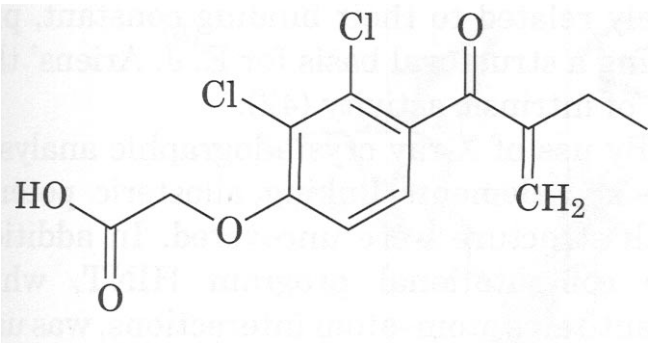
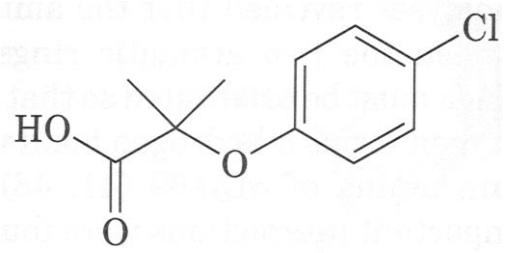


(2) ethacrynic acid



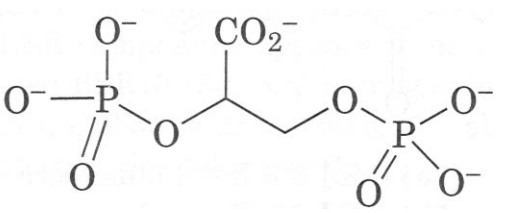
(3) clofibric acid

concentrations of ethacrynic acid were needed to interact with Hb in deformed red cells (27). Clofibric acid, when administered in a 2 gm/day dose (as the ethyl ester clofibrate), appeared to be an ideal potential treatment for sickle-cell anemia, but was subsequently found to be highly bound to serum proteins and not transported in quantities sufficient to interact with sickle Hb. Furthermore. structure-based derivatives were not found to be effective (28, 29).

The major problem with designing a small molecule to treat sickle cell anemia is not so much an issue of specificity, but arises from the treatment of a chronic disease. The potential cumulative toxicity from the amount of drug needed to interact with approximately two pounds of hemoglobin S over a homozygous patient's lifetime is the major concern (22) (for a review, see Vol. 3, Chapter 10. *Sickle* ***Cell Anemia,*** by Alan Schecter et al).

2.2!.3 *Allosteric* Effectors. *2,3-Diphospho*-glycerate (2,3-DPG, compound 4), found in most mammalian red cells, is the naturally occurring allosteric effector for Hb. Its physiological role is to right shift the Hb oxygen-binding curve to release more oxygen. The binding site of 2,3-DPG, determined by Arnone (30) lies on the dyad axis at the mouth of the β-cleft (Fig. 10.2) interacting with the N-terminal βVal1, βLys82, and βHis143 of deoxy Hb. A more recent study at a higher resolution, by Richard et al. (31), found DPG to interact with the residues βHis2 and βLys82. Goodford and colleagues were the first to design agents that would bind to the 2,3-DPG site (32–34). An effective allosteric effector that can enter red cells might be used to treat hypoxic diseases such as angina and stroke, to enhance radiation treatment of hypoxic tumors, or to extend the shelf life of stored blood.

Many antigelling agents left shift the oxygen binding curve, producing higher concentrations of oxy-HbS. Given to patients with sickle-cell anemia, this should result in less polymerization, and therefore less red blood cell sickling. It was a surprise therefore when clofibric acid, which blocks sickle-cell Hb polymerization, was found to shift the Hb oxygen binding curve to the right, in a manner similar to that of 2,3-DPG (25). The clofibric acid binding site was found to be far removed from the 2,3-DPG site (25, 35). The determination of the clofibric acid binding site on Hb was the first report of a tense state (deoxy state) allo-



(4) 2,3-DPG

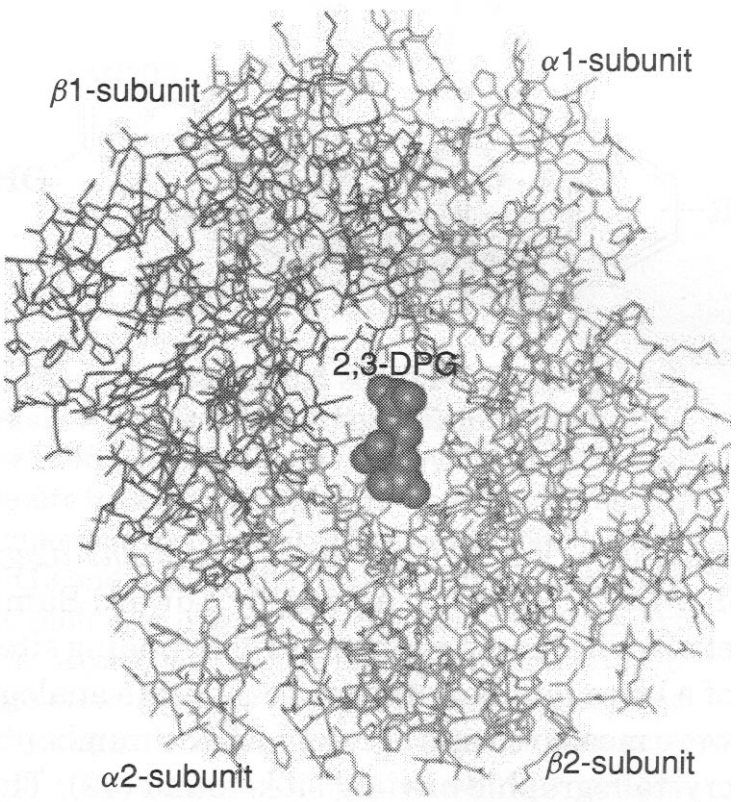


Figure 10.2. View of (4)(2,3-DPG) binding site at the mouth of the β-cleft of deoxy hemoglobin. See color insert.