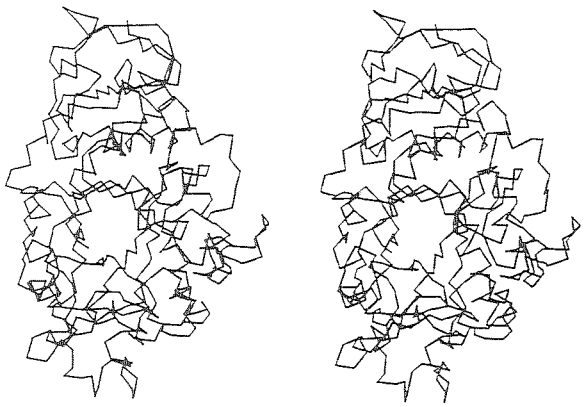
substrates and inhibitors, namely, maltotriose, maltose, glucose and four kinds of iodine derivatives of substrates. These D-Fourier maps showed two main binding sites I and II. The site II is at the bottom of the cleft in the main domain and also located at the C-terminal end of the β-barrel. The site I is at one of the four S-S bridges and about 30.0 A away from the site II along the molecular surface. There are not binding sites between the site I and II. The D-Fourier map for the PCMB complex crystal prepared in the presence of EDTA showed that the PCMB binds to the site close to the S-S bridge at the site I. PCMB is reported to bind to the SH-group of the enzyme which is one of chelating groups to the essential calcium ion. His and Asp are speculated to be catalytic residues judging from the pH dependence of the enzyme activity. The two residues at the edge of the site II seems to be assigned as His and Asp referring to the e.d. map, the primary structure and heavy atom binding.



02.1-33 PORCINE PANCREATIC α-AMYLASE : CRYSTAL STRUCTURE AND BINDING SITES. By G. BUISSON, E. DUEE (D.R.F., C.E.N., 85 X, 38041 Grenoble cedex, France) and R. HASER, F. PAYAN, N. DARBON (CRMC^2, Campus de Luminy, case 913, 13288 Marseille cedex 9, France).

The crystal structure study of porcine pancreatic α-amylase I (Mr∿53000) has now been extended to 2.9 Å resolution. The electron density map is currently being interpreted and the earlier conclusions inferred from the 5 Å resolution model are already confirmed.

Among the prominent features of the molecule are the predominance of extended chain conformation and the large cleft previously identified as the active site region (Payan et al., Acta Cryst. (1980) B36, 416-421). There is no obvious division of the molecule into two identical subunits as proposed by Robyt et al (Arch. Biochem. Biophys. (1971) 144, 160-157) or into two similar structural domains as suggested by Fitzgerald et al (J. Mol. Biol. (1979) 135, 753-756). A number of additional interesting features will be discussed in the light of known properties of the enzyme, current sequence work (Pasero et al., Biochimie (1981) 63, 71-76) and previous proposals about its catalytic activity :

- The Cl^- ion, which is known to activate the enzyme (Levitzki and Steer, Eur. J. Biochem. (1974) 41, 171-180), binds in a pocket near the active site cleft.

- The edge of this cleft provides also the region involved in binding one calcium ion. Since this calcium ion is easily replaced by a strontium one it may not be the firmly bound and essential ion for the enzymic activity (Vallee et al., J. Biol. Chem. (1959) 234, 2901-2929 ; Levitzki and Steer (1974)). However, no other calcium ion site has been located so far.

- Substrate analogues bind to two different regions of the molecule : one corresponds to the active site cleft, the other is located about 30 Å from this crevice and on the surface of the molecule. The latter may function as a storage or a regulation site. In this respect, it is noteworthy that bile salts, which act as effectors of the amylase activity (O'Donnel et al., Enzymes (1975), 19, 129-139), bind only to this second site. If one will confirm that this secondary substrate-binding site has a regulatory function, its importance for the mechanism of α-amylase action must be stressed.

02.1-34 MALTOHEPTAOSE BINDING TO PHOSPHORYLASE a AT 0.25 nm RESOLUTION. By E. Goldsmith, S. Sprang, R.J. Fletterick, Dept. of Biochemistry & Biophysics, University of California, San Francisco, CA 94143.

Glycogen activates glycogen phosphorylase through binding at a site distant from the catalytic site. This interaction has been modeled by crystallographic analysis at 3.0 Å of maltoheptaose, bound to phosphorylase a (Kasvinsky et al., J. Biol. Chem. (1978) 253, 1290-1296) and of maltotriose bound to phosphorylase b (Weber et al., Nature (1978) 274, 433-437). We have extended the resolution of the first study to 2.5 Å, and have included use of partially refined phases. The improved resolution has permitted the determination of the structure of maltoheptaose as it is bound to the enzyme, and has allowed analysis at the interactions with the protein and the protein conformational changes.

The maltoheptaose conformation is a left-handed helix with helical parameters close to those observed in small molecule crystal structures and estimated for amylase. The H-bond O(2)→O(3') found in related small molecules is preserved (Quigley et al., J. Amer. Chem. Soc. (1970) 92, 5834-5839).

Two molecules of maltoheptaose bind. One of these is well-localized and all 7 glycopyranosides are observed to some extent. A second molecule is bound (to site E of ref. 1) with only 2 sugars localized. The orientation of this sugar puts the reducing O(1) near the O(6) of the first ring of the tightly-bound maltoheptaose. The relative orientation suggests that phosphorylase binds α(1-6) branch points on glycogen, and further that it binds β-chains rather than loose ends.

The maltoheptaose makes numerous H-bonding interactions with the protein to ionic and polar side chains, causing local conformational changes, and changes at the active site.