Photogenic impurities

from Henry Slayter

TWO papers, published together in the *Journal of Biological Chemistry* (**254**, 1747; 1979) come essentially to the same conclusion concerning the structure of pyruvate carboxylase examined by electron microscopy. Goss *et al.* of the University of Adelaide and Cohen *et al.* of Case Western Reserve and the National Institute for Medical Research, London, have both applied negative staining to this molecule, obtaining different results from those previously reported by Valentine *et al.* (*Biochemistry* **5**, 3111; 1966) using the same technique. (Two of the coauthors of the latter group were also coauthors of the original Valentine group.)

Negative staining was first described and applied by C.E. Hall and later applied skillfully and to great advantage by many investigators (including, especially, Valentine and R.W. Horne) in the study of biopolymeric structures. In this method, the purified preparation is mixed with concentrated electron-dense 'stain', usually a 1 — 5% solution of uranyl acetate, uranyl formate, sodium phosphotungstate or sodium phosphomolybdate. The preparation is air-dried to form a thin layer supported on 10-nm carbon films. Stain, which is excluded by biopolymer components, penetrates voids, thus outlining the structure. Electron contrast results from the greater electron scattering power of the stain (average density about 7) compared with that of the specimen.

Inherently, this technique is a simple and effective method for examining macromolecules larger than about 5 nm in diameter — but pitfalls abound. Resolution is limited by granularity of the stain, alteration of specimen structure by dehydration, extent to which contrast can be induced in small subunits, and, to an indeterminate extent, specimen damage in the electron beam. Other difficulties relate to the effects of these rather unusual chemical salts on the specimen (due to *p*H and ionic strength requirements, the reducing nature of the salts, the presence of divalent cations and so on). Penetration of the specimen by stain can produce misleading measurements of dimensions or loss of fine structure. As in any method for the examination of individual macromolecules, absolute specimen purity is vital.

Valentine *et al.* originally described the pyruvate carboxylase molecule as a distinctive square-planar tetramer of globular subunits. Several lines of evidence supported this premise. The highly photogenic square-planar configuration appeared in enzyme preparations isolated from several animal species. Dimensions obtained by electron microscopy were consistent with the measured molecular weight of 500,000, consisting of four similar or identical subunits of molecular weight 125,000. The square-tetramers exhibited cold-lability, a well characterised property of chicken liver pyruvate carboxylase.

Valentine, however, also showed that the same enzyme from yeast had a rhombic appearance (Valentine, *Proc. fourth Regular Conf. (Europe) on Electron Microscopy, Rome* **2**, 3; Tipografia Poliglotta Vaticana, Rome, 1968). Now, the new reports show that a similar structure characterises pyruvate carboxylases of animal origin, and that the square-planar molecule is a contaminant commonly found in such preparations. That this contaminant was so much more evident than the enzyme itself is explained, in part, by the demonstration of Goss *et al.* that this structure is actually an octamer in which the thickness (and therefore the relative contrast) of each subunit is doubled.

Both new studies confirm that pyruvate carboxylase consists of four subunits. Cohen *et al.* suggest a rhombic structure with major and minor dimensions of 19 nm and 16 nm respectively, and a centre-to-centre distance between adjacent subunits of 7.3 nm. (They report that exact dimensions vary by as much as 10 % depending on the substance used as negative stain.) Goss *et al.* describe the molecule as a 'splayed tetrahedron' with a long axis of approximately 17.7 nm and a short axis of 15.2 nm with centre-to-centre distance again 7.3 nm. The main difference between the two interpretations is whether the subunits lie in a plane (rhombic structure) or are without regular mutual separation and are not coplanar (splayed tetrahedron).

Cohen *et al.* indicate that their data cannot be fully interpreted with regard to the question of subunit arrangement in three dimensions, but Goss *et al.* suggest that one pair of subunits is displaced slightly above the plane of the second pair. Examination of the field of negatively stained molecules presented by the latter authors shows that only a small number of images can be so interpreted. Thus, while their speculative model is most interesting, I must admit difficulty in accepting with certainty either the proposed three-dimensional arrangement or the detailed tear-drop model of subunits. Further understanding of the structure of this enzyme must await preparation of crystals and their examination by iterative electron microscopy or X-ray crystallography.

Anti-biotin antibody and pyruvate carboxylase are known to form soluble complexes. Cohen *et al.* also present micrographs of this complex, which indicate that the antibody binds to the surface of the tetramer at the same general location as does avidin. Thus, additional evidence is presented that the rhombic tetramers are in fact a biotin-containing protein.

In perspective: some will remember that similar interpretive difficulties have arisen in past studies of negatively stained enzyme preparations — brought on, likewise, by impurities of a significantly more photogenic character than the plainer principal subject. (DNA-dependent RNA polymerase, first reported to be amorphous globular protein, was credited by a second group with a distinctive polyhedral structure. A third group showed up the polyhedron as a mere contaminant and found the polymerase to be an amorphous globular protein.)

What can be learned from all this — perhaps — is that while carefully applied electron microscopy can be of tremendous and unique importance in providing structural information, interpretation must always be carried out with extraordinary care. Awareness of the artefacts to which biopolymers are prone is a must, and investigations should include parallel studies in solution which pose the same questions asked of the electron microscope. Above all, very careful attention must be given to the purity of the preparation. Even a few per cent of contaminating molecules, especially those of the more photogenic sort, can — given the harsh treatment inherent in this method — be interpreted as being a few per cent of survivors of the structure sought. □

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Budgeting for volcanoes

from Peter J. Smith

WHAT is the energy budget of a volcano? Or to be more explicit, what proportions of a volcano's energy are carried away by the various modes of energy transport? What, for that matter, are the appropriate transmission mechanisms? These are the sort of conceptually simple questions one would expect some nineteenth century volcanologist to have answered, albeit incorrectly; but whether he did or not, the same questions are still being asked in 1979, possibly because they are not as easy to come to terms with as might be supposed. Certainly they are not answered entirely satisfactorily by McGetchin and Chouet (*Geophys. Res. Letts.* **6**, 317; 1979) even now, as these authors freely admit.

But to start at the beginning, for many years McGetchin and Chouet have been studying the behaviour of Stromboli, the famous volcanic island that rises about 920m above the Mediterranean some 75km

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