

of TN-C were identified that bound to TN-I. This is in agreement with similar work done on TN-C fragments by Weeks and Perry (*Biochem. J.*, **173**, 449; 1978) and also with earlier work done on TN-I.

At the same time the work of Leavis *et al.* revealed a limitation of looking at fragments of TN-C. It was found that fragments containing the two  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$  sites, regions III and IV, had a strong affinity for  $\text{Ca}^{2+}$  and could also bind  $\text{Mg}^{2+}$ . Further cleaving the polypeptide, so that regions III and IV were on separate fragments, resulted in the isolated  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$  binding sites changing their characteristics. Now they could only bind  $\text{Ca}^{2+}$  weakly and failed to bind any  $\text{Mg}^{2+}$  at all. This illustrates the importance of tertiary structure (relationships between different regions of the same molecule) for defining the properties of the binding sites. Indeed, the  $\text{Ca}^{2+}$ -binding properties of TN-C are related to its quaternary structure (relations to surrounding protein molecules), because when TN-C binds to TN-I, its affinity for  $\text{Ca}^{2+}$  increases by an order of magnitude.

In order to obtain a better understanding of how  $\text{Ca}^{2+}$  binding to TN-C releases the inhibitory effect of the troponin complex and tropomyosin, it is crucial to know which  $\text{Ca}^{2+}$  binding sites on TN-C are the regulatory ones. The bulk of the evidence suggests that it is the  $\text{Ca}^{2+}$ -specific sites that regulate contraction. Work with myofibril preparations indicate that the  $\text{Ca}^{2+}$  concentration at which their ATPase rate is activated is independent of the concentration of  $\text{Mg}^{2+}$ . Hence, only the  $\text{Ca}^{2+}$ -specific sites are involved in regulation; but work on the tension development in muscle suggests that raising the concentration of  $\text{Mg}^{2+}$  increases the  $\text{Ca}^{2+}$  concentration required for the muscle to be activated. This possibly implicates the  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$  sites in regulation.

The solution of this dilemma would be an important advance in understanding how TN-C regulates contraction. A crucial piece of data relevant to this question is the concentration of  $\text{Mg}^{2+}$  in muscle. This is not accurately known, but it is believed to be in the range 0.1 to 5mM. This knowledge would enable the metal ions bound to TN-C to be identified in contracting and relaxed muscle. When the  $\text{Ca}^{2+}$  concentration is high and the muscle is contracting, all four sites on TN-C bind  $\text{Ca}^{2+}$ . However in relaxed muscle when the  $\text{Ca}^{2+}$  concentration is low, the  $\text{Ca}^{2+}$ -specific sites are free of  $\text{Ca}^{2+}$ , while the  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$  sites could be binding  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , depending upon the concentration of  $\text{Mg}^{2+}$ . There is also much more to learn about the relationships between TN-C and its

neighbours TN-I and TN-T. For it is these changing relationships, as TN-C binds and releases  $\text{Ca}^{2+}$ , that release and trigger the inhibitory action of troponin and tropomyosin.

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## Nuclear resonance & synchrotron radiation

*from G. V. Marr*

THE electromagnetic radiation (synchrotron radiation) emitted by high energy electrons when they are constrained by magnetic fields to move in circular orbits in an electron synchrotron or storage ring is now well known and much used as an intense source of ultraviolet and X-ray photons. Because the electrons are injected as localised bunches into the synchrotron and are accelerated to speeds approaching the speed of light, the synchrotron radiation emitted is highly polarised and concentrated into tightly collimated beams forward-directed along tangents to the instantaneous electron orbit. The radiation emitted is characterised by the electron energy and the bending radius of the orbit so that its spatial and energy distribution is calculable. It appears as a continuum of radiation extending from the infra-red to the X-ray region with a peak in the XUV or X-ray region. It also possesses a pulsed-time structure determined by the orbiting frequency of the electron bunches. There are now more than 20 laboratories throughout the world, 7 of which are in Western Europe, where facilities exist for the exploitation of synchrotron radiation and research programmes are in operation to study problems in atomic, molecular, surface and solid state physics, in chemistry, and in the structural properties of solids and biological samples. In addition, X-ray microscopy, lithography, the calibration of light sources and detectors, etc., are applications of synchrotron radiation.

New applications are continuously appearing and an interesting extension of these activities to the observation of nuclear resonance excitation has been reported by R. L. Cohen *et al.* (*Phys. Rev. Letters* **41**, 381; 1978). The interest is not so much that new data on the nucleus has been revealed, indeed the observations are of the well known 14 keV level of  $^{57}\text{Fe}$ , but that the apparatus developed enables the resonance to be detected by the observation of a weak flux of conversion electrons emitted by the decay of the nuclear state in the presence of a much more intense electron flux from non-

nuclear electronic processes.

The method makes use of the fact that the University of Stanford storage ring can provide pulses of synchrotron radiation 0.3 ns wide every 780 ns. With a long lifetime (100 ns) against decay of the nuclear level it has been possible to gate the detector system so as to collect data 100 ns following the incidence of the synchrotron radiation pulses, thereby allowing photoelectrons, Compton electrons and long lived secondary electrons to dissipate before the detector is activated.

The synchrotron radiation was dispersed by a crystal X-ray monochromator to provide a band width of 3 eV photon energy centred about the 14 keV excitation energy. This radiation fell onto a  $^{57}\text{Fe}$  enriched iron foil and the emitted electrons entered a continuous semi-conducting dynode electron multiplier which was divided into two stages with a gated grid between the two sections. The grid was gated "off" during the prompt photoelectron pulse and so removed the main cause of afterpulsing electrons which would be otherwise indistinguishable from the nuclear conversion electrons. Very low energy electrons having flight times of as long as 100 ns were removed at the electron multiplier entrance.

Under these conditions, the crystal monochromator was scanned over the energy of the  $^{57}\text{Fe}$  nuclear resonance and the counting rate plotted as a function of X-ray energy. The width of the resonance is negligible ( $5 \times 10^{-7}$  eV) compared with the band width of the monochromator so that the scan actually profiled the transmission function of the monochromator and the width and shape of the response reflect the monochromator output. Nevertheless the resonance was observed to occur at exactly the anticipated energy and was of the correct intensity. The authors also point out that their detector system can be used with advantage to provide a study of the Bragg-scattering profile of crystal monochromators in synchrotron radiation beam lines, thus allowing maladjustment of the monochromator or defining slits to be corrected *in situ*.

New synchrotron radiation sources are being designed specifically to make use of the synchrotron radiation rather than to provide high energy particles. In particular, the new 2 GeV storage ring under construction at the Daresbury Laboratory in the U.K. will be used to study the time structure of the radiation. Looking further ahead, the European Science Foundation is currently considering the design requirements for a European Synchrotron Radiation Facility for the 1980s.

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