

the substrate alone, but in the presence of the totally inactive trypsin-inhibitor complex, a re-activation of trypsin and neutral proteinase activity was observed. This can only be accounted for by the replacement of trypsin and neutral proteinase in the enzyme-inhibitor complex by enzymically inactive Tos-Phe-CH₂Cl-chymotrypsin.

Chymotrypsin and its precursor chymotrypsinogen have no proteolytic activity on fluorescein-labelled polymeric collagen fibrils that can be assayed by direct fluorimetry of solubilized peptides. Yet chymotrypsinogen can be shown to be capable of re-activating trypsin and neutral proteinase that have previously been completely inactivated by the tumour inhibitor (Fig. 2). In an exactly similar manner, Trasylol-trypsin (having no proteolytic activity on the substrate alone) releases trypsin and neutral proteinase previously bound in their enzyme-inhibitor complexes. In these two cases, the regain of proteolytic activity is a time-dependent process (Fig. 2) dependent upon the concentration of added competitor.

Clearly the enzyme-inhibitor complex can be dissociated by suitable donors of disulphide groups located at a site other than the active centre of these enzymes. These data indicate an allosteric control of certain proteolytic activities mediated by thiol-disulphide exchange.

Hummel, B. C. W. (1959) *Can. J. Biochem.* **37**, 1393-1399

Steven, F. S. (1976) *Biochim. Biophys. Acta* **452**, 151-160

Steven, F. S., Podrazký, V. & Itzhaki, S. (1977) *Biochim. Biophys. Acta* **483**, 211-214

Amino Acid Compositions Provide a Reliable Guide to Sequence Similarities

ATHEL CORNISH-BOWDEN

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Although several indexes have been proposed for comparing the amino acid compositions of proteins of unknown sequence (Metzger *et al.*, 1968; Harris *et al.*, 1969; Marchalonis & Weltman, 1971; Dedman *et al.*, 1974), the usefulness of all of these has been severely limited by the absence of any theory that might permit reliable information about sequence similarity to be deduced. All of them are highly dependent on the lengths of the proteins compared, and no valid information can be obtained from them if the length-dependence is ignored. In an effort to overcome these difficulties, I proposed (Cornish-Bowden, 1977) that the number of differences between the sequences of two proteins A and B might be estimated from the following formula:

$$S\Delta n = \frac{1}{2} \sum (n_{iA} - n_{iB})^2$$

in which n_{iA} and n_{iB} are the numbers of residues of the i th type of amino acid in proteins A and B respectively, if both proteins have the same total number of residues, N , and the summation is carried out over the 18 types of amino acid commonly distinguished in composition measurements. According to a simple theory, $S\Delta n$ is an unbiased estimator of the number of sequence differences, with a predicted coefficient of variation of about 38%; a value of $S\Delta n$ less than $0.42N$ indicates significantly greater similarity than would be expected from chance alone. The usefulness of this theory clearly rests on whether the simple assumptions that it incorporates are valid for real proteins. This can be tested by calculating $S\Delta n$ for pairs of proteins of known sequence and comparing the results with the actual numbers of sequence differences.

To test the validity of the significance test, 83 proteins with different names and known sequences were chosen from Dayhoff (1972, 1973). These were arranged in order of increasing size to give a series with N ranging from 24 to 374. Each protein was then compared with its nearest and next-nearest neighbours in the series. (In doing this the

requirement that each comparison be between two proteins with the same total number of residues was relaxed to a requirement that the numbers do not differ by more than a few per cent.) In all there were 163 comparisons, of which seven showed significantly similar compositions. All of these seven showed significant sequence similarity when the sequences were examined. Of the remaining 156 comparisons for which significant composition similarity was not seen, 144 showed no significant similarity of sequences either. So the test gave the correct result in more than 90% of cases and, more important, in no case did it indicate similarity when none existed in the sequences.

To test the validity of $S\Delta n$ as an estimator of the number of sequence differences between a pair of related proteins, all possible pairs of known cytochrome *c* sequences were examined. Averaged over 1830 comparisons, $S\Delta n$ was found to be slightly biased, estimating about 13% more sequence differences than there were in fact, with a coefficient of variation of 43%. Similar results were obtained with other sets of proteins, including insulin, the snake venoms, ribonuclease, crystallin and various globins. In all cases the observed behaviour of $S\Delta n$ was similar to that predicted.

Cornish-Bowden, A. (1977) *J. Theor. Biol.* **65**, 735-742

Dayhoff, M. O. (1972) *Atlas of Protein Sequence and Structure*, vol. 5, National Biomedical Research Foundation, Silver Spring, MD

Dayhoff, M. O. (1973) *Atlas of Protein Sequence and Structure*, vol. 5, suppl. 1, National Biomedical Research Foundation, Silver Spring, MD

Dedman, J. R., Gracy, R. W. & Harris, B. G. (1974) *Comp. Biochem. Physiol. B* **49**, 715-731

Harris, C. E., Kobes, R. D., Teller, D. C. & Rutter, W. J. (1969) *Biochemistry* **8**, 2442-2454

Marchalonis, J. J. & Weltman, J. K. (1971) *Comp. Biochem. Physiol. B* **38**, 609-625

Metzger, H., Shapiro, M. B., Mosimann, J. E. & Vinton, J. E. (1968) *Nature (London)* **219**, 1166-1168

The 0-, 4- and 6-Sulphated Disaccharides of Chondroitin Sulphates: Their Electrophoretic Separation and Detection with *p*-Anisidine

ROBERT J. ELLIOTT and DUGALD L. GARDNER

Department of Histopathology, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

Chondroitin sulphates are depolymerized by chondroitin ABC lyase (EC 4.2.2.4) to yield unsaturated sulphated disaccharides 2-acetamido-2-deoxy-3-*O*-(β -D-glucopyranosyluronic acid)-4(or -6)-*O*-sulpho-D-galactoses and from chondroitin 4-sulphate or chondroitin 6-sulphate respectively (Saito *et al.*, 1968; Thurston *et al.*, 1975). After separation by chromatography or electrophoresis, the sulphated disaccharides have been identified with alkaline AgNO₃ (Suzuki *et al.*, 1968; Trevelyan *et al.*, 1950). This reagent can detect approx. 25 μ g of sulphated disaccharide but produces some diffusion of the spots. This report describes the use of *p*-anisidine (*p*-methoxyaniline) as a coloured detection probe for nano- and micro-gram quantities of chondroitin sulphate disaccharides after separation by paper electrophoresis.

Human articular-cartilage chondroitin sulphate and purified standards were incubated with chondroitin ABC lyase (Seikagaku Kogyo Co., Tokyo 103, Japan) in 0.1M-acetate/Tris buffer, pH 7.90, at 37°C for 90 min (Saito *et al.*, 1968). Some samples were subsequently treated with chondro-4-sulphatase to remove the sulphate from the 4-sulphated disaccharide (Suzuki *et al.*, 1968). The electrophoretic separation of un-sulphated and 4- and 6-sulphated disaccharides was on Whatman no. 1 chromatography paper (32cm \times 17cm). Samples (5 μ l) were applied to the buffer-saturated paper 6cm from the cathode, permitting a migration distance of 18cm to the anode. The electrophoresis buffer was 0.05M-citrate, pH 4.00 (Hata & Nagai, 1972). Separation of the disaccharides was obtained by using 20mA (200-300V) for 4.5h. The paper was then dried at 75°C for 1h. The detection reagent was prepared by dissolving 1g of *p*-anisidine/