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Porath J, Axén R & Ernback S. Chemical coupling of proteins to agarose.
Nature 215:1491-2, 1967.
[Institute of Biochemistry, University of Uppsala, Sweden]

In alkaline media, cyanogen bromide reacts with hydroxyl groups of agarose and other polysaccharides. The polymers are converted to highly reactive derivatives. Such 'activated gel derivatives' are then used to prepare a variety of adsorbents for bioaffinity chromatography, diagnostics, and immobilized enzymes. [The *SCI*[®] indicates that this paper has been cited in over 860 publications since 1967.]

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"As a continuation of our developmental work on molecular sieving gels (Sephadex, etc.), Per Flodin and I, together with Lennart Rhodén, attempted to prepare biospecific adsorbents for isoagglutinins. Isocyanate groups were introduced into Sephadex to make possible thiourea bridging with blood group substances. Due to unforeseen circumstances, the work was interrupted. Several years later, Rolf Axén and I published a similar immobilization procedure¹ which paved the way for the introduction of the first gel-based radioimmunoassay technique.² We soon realized, however, that a different, and perhaps better, immobilization technique was highly desirable to improve and extend our newly introduced method.

"Axén joined Bernhard Witkop at Bethesda to learn new techniques for selective cleavage of protein. Upon his return to the Institute of Biochemistry at Uppsala, he suggested a new approach to our project, *viz.*, the coupling of substances containing primary amino groups to cyanamide-Sephadex. The idea was suggested as a research project to Sverker Ernback, one of our first-year research students. The cyanamide-Sephadex was prepared by treating amino-Sephadex with cyanogen halide. To my surprise, the yield of coupled amino acid exceeded 100

percent! I urged Ernback to make blind experiments using unsubstituted Sephadex. Indeed, Sephadex after cyanogen halide treatment was found to immobilize protein in excellent yields!³

"Cyanogen bromide coupling replaced the isothiocyanate procedure for the synthesis of immobilized antigens and antibodies to be used in radioimmunoassays. It is still the most commonly used method for preparing gel- and paper-based immunodiagnoses (RIA, RAST, PRIST, etc.).

"Our discovery of the cyanogen halide coupling method was followed by hectic work in several directions. Enzymes and enzyme inhibitors were immobilized onto a variety of hydroxylic supports. Not much later, our work on activation of agarose for enzyme immobilization was described, and this work initiated an almost explosive development in (bio-)affinity chromatography.

"We interpreted the activation to involve the formation of cyanate followed by its rapid conversion to imino carbonate. The final coupling products were thought to be gels containing mixtures of imino carbonic acid esters, carbonic acid esters, and carbamate substituents, and, somewhat later, isourea linkages were also considered. Evidence for this interpretation was obtained from IR-spectra including also some model compounds.

"The complicated scheme of reactions is now fairly well understood, thanks to Meir Wilchek⁴ and others. The nucleophilic displacement of the ligands with ammonia is particularly interesting: original amino groups are converted into guanidino groups. By using the cyanogen bromide activated support as an organic reagent, Wilchek converted insulin into 'superinsulin.'

"Our original suggestions have been essentially confirmed, but the recent work has shed light on some important limitations. In improved form, the cyanogen bromide coupling is still the preferred method for the preparation of most biospecific adsorbents used in (bio-)affinity chromatography, and agarose is by far the most commonly employed support."

1. Axén R & Porath J. Chemical coupling of amino acids, peptides and proteins to Sephadex. *Acta Chem. Scand.* 18:2193-5, 1964. (Cited 35 times.)
2. Wide L & Porath J. Radioimmunoassays of proteins with the use of Sephadex-coupled antibodies. *Biochim. Biophys. Acta* 130:257-60, 1966. (Cited 570 times.)
3. Axén R, Porath J & Ernback S. Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* 214:1302-4, 1967. (Cited 1,655 times.)
4. Kohn J & Wilchek M. The determination of active species on CNBR and trichloro-s-triazine activated polysaccharides. (Gribnau T C J, Visser J & Nivard R J F, eds.) *Affinity chromatography and related techniques: theoretical aspects/industrial and biomedical applications*. Amsterdam: Elsevier Scientific Publishing, 1982. p. 235-44.