Journal of Solid-Phase Biochemistry, Vol. 4, No. 3, 1979

SOME RECENT DEVELOPMENTS IN GENERAL LIGAND AFFINITY CHROMATOGRAPHY[1]

KLAUS MOSBACH

Biochemistry 2,
Chemical Center,
University of Lund, P.O. Box 740,
S-220 07 Lund 7, Sweden

Accepted May 22, 1979

The use of immobilized adenine coenzymes as general ligands in affinity chromatography has received considerable attention during the last few years. Almost one-third of the enzymes (about 2000) for which a specific number has been assigned require participation of one of the four adenine coenzymes: NAD, NADP, CoA, and ATP. The use of such versatile ligands with a broad biological affinity-binding spectrum has a considerable advantage, since a new synthetic route does not have to be devised for every putative biospecific purification. Since a review on the topic of "general ligand affinity chromatography" has recently appeared (1), I will briefly discuss in this report only some new techniques in this area that have been studied recently by our group.

HIGH PERFORMANCE LIQUID AFFINITY CHROMATOGRAPHY (HPLAC)

By covalent coupling of AMP or an antigen, e.g., anti-human serum albumin, to a support material such as glycosil and applying these in high performance liquid chromatography, it was possible to introduce bioaffinity into these systems (2). Thus, enzymes/isozymes and albumins, respectively, were successfully separated. This combination of "conventional" HPLC technique with that of affinity chromatography should find wide application in various areas, including the clinical, where there is a need for rapid separations of biological macromolecules. Compared to stationary phases used in conventional high performance liquid chromatography, the affinity

[1]This paper was presented at the symposium on "Hydrophobic and Biospecific Affinity Chromatography" held at Ruhr-Universität Bochum, West Germany, October 4, 1978.

0146-0641/79/0900-0167$03.00/0 © 1980 Plenum Publishing Corporation

ligand introduces a far higher specific component in the separation step, which therefore could reduce the need for prepurification of samples. Compared with affinity chromatography, this technique is much faster, and should be useful not only for routine separation and enrichment of macromolecules, but also in their analysis in biological samples. It is conceivable that even a better resolving power with this technique will be observed compared with affinity chromatography in both the adsorption and elution steps.

MAGNETIC AFFINITY CHROMATOGRAPHY

Recently, magnetic affinity supports, including enzyme affinity ligands and immunosorbents, have been developed (see, e.g., 3–6). Such preparations allow convenient retrieval of affinity bound biomolecules even from crude samples. We have recently used in enzyme purification Sepharose-bound AMP that had been turned "magnetic" on contact with a solution of ferrofluid. Using these preparations, alcohol dehydrogenase could be obtained in pure form within half an hour from a crude extract of horse liver, obviating the need for centrifugation and column separation.

AFFINITY PRECIPITATION OF ENZYMES

A bifunctional nucleotide derivative consisting of two NAD moieties connected by a spacer has been synthesized (7). This compound has been applied as precipitating agent for enzymes, and the process is similar to the precipitation technique known for antibodies. The enzyme studied was tetrameric lactate dehydrogenase, which could be efficiently precipitated from a solution containing the above mentioned bis-NAD and pyruvate, the latter compound allowing ternary complex formation to take place, thereby strengthening the binding and assuring a high degree of enzyme specificity. It is likely that affinity precipitation of enzymes will be useful not only in analysis and enzyme purification, but also in morphologic and topographic studies on enzymes.

ENZYMIC REGENERATION OF NAD COVALENTLY BOUND TO ALCOHOL DEHYDROGENASE

The use of immobilized coenzymes as components of coenzyme dependent enzymic systems for the synthesis and analysis of a variety of

compounds is well established (1). Brief mention should be made here to a specific approach to this problem (8). Instead of allowing the coenzyme, in this particular case NAD, to "oscillate" between its site of "consumption" and that of "regeneration," it was bound covalently directly to the enzyme in such a way that it both could interact with the enzyme to which it was bound and subsequently "flip out" from the enzymes' active site to be regenerated by a second enzyme. The system prepared was an NAD-liver alcohol dehydrogenase complex, which was recycled by beef heart lactate dehydrogenase. A recycling rate of $\sim 300\,h^{-1}$ was obtained. Such "self-contained" catalytic units with NAD permanently fixed as a prosthetic group could be of value for applications in enzyme reactors, analytical devices such as "reagent-less" electrodes, or for the treatment of enzyme deficiency diseases involving coenzyme-dependent enzymes.

REFERENCES

1. MOSBACH, K. (1978) *In* Advances in Enzymology, Vol. 46, MEISTER, A. (ed.), Wiley, New York, p. 205.
2. OHLSON, S., HANSSON, L., LARSSON, P.-O., and MOSBACH, K. (1978) FEBS Lett. 93 : 5.
3. MOSBACH, K., and ANDERSSON, L. (1977) Nature 270 : 259.
4. GUESDON, J. L., and AVRAMEAS, S. (1977) Immunochemistry 14 : 443.
5. KRONICK, P. L., CAMPBELL, G. L., and JOSEPH, K. (1978) Science 200 : 1074.
6. DUNNILL, P., and LILLY, M. D. (1974) Biotechnol. Bioeng. 16 : 987.
7. LARSSON, P.-O., and MOSBACH, K. (1979) FEBS Lett. 98 : 333.
8. MÅNSSON, M.-O., LARSSON, P.-O., and MOSBACH, K. (1979) FEBS Lett. 98 : 309.