# THE IDENTIFICATION AND ISOLATION OF LACTOPEROXIDASE FROM SALIVARY GLAND

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There are numerous problems involved in establishing a direct relationship between peroxidase activity of cells and cell extracts and the presence of enzymatic peroxidases. The presence of catalase in relatively high concentrations in tissues makes the usual peroxidase assay of limited value, since low values may result because of its destruction of peroxide. On the other hand, metallo proteins such as ceruloplasmin, ferritin, hemoglobin, myoglobin, and the cytochromes will all give peroxidase activity. Even though these compounds themselves actually have relatively low specific peroxidase activity, their presence in high concentrations may give misleading results.

Immunochemical methods, which are very specific, can be used independently to establish the presence of a particular protein. The mammalian peroxidase, bovine lactoperoxidase, has been found to be antigenic in the rabbit. It is, therefore, possible to produce antibodies specific for the purified enzyme, lactoperoxidase (Allen and Morrison, 1963).

It is the object of the present report to present, in preliminary form, the results of immunodiffusion analyses, using various tissues and tissue extracts.

## METHODS AND RESULTS

Purified lactoperoxidase was prepared as previously described (Morrison and Hultquist, 1963). Peroxidase assays and protein analyses were carried out as previously indicated (Morrison and Hultquist, 1963; Allen and Morrison, 1963). Antisera to the purified enzyme were prepared in rabbits by intramuscular injection of 1 ml of 1 mg of enzyme protein per ml of complete Freunds adjuvent at weekly intervals for three weeks. The animal was bled three weeks after the last injection. Agar diffusion was carried out as previously described (Allen and Morrison, 1963). Immunodiffusion methods employed could readily detect 10-15 γ/ml of lactoperoxidase.

Bovine tissues were freed of fat and connective tissue when possible. Each gram of tissue was homogenized with one ml of 0.1 M phosphate buffer, pH 7.4, containing one percent sodium cholate. The homogenate was centrifuged at 10,000 g for two hours. The clear supernatant fluid was then employed directly for immunodiffusion or was dialyzed and concentrated by lyophilization prior to analysis.

As can be seen in Figure 1, a band which showed complete fusion with the purified enzyme was given by bovine salivary gland extract. No other tissue gave such a band, even when the protein concentration was increased ten or twenty fold.

To further establish the presence of the enzyme in salivary gland, the enzyme was isolated by a modification of the method employed for milk (Morrison and Hultquist, 1963). The enzyme obtained in this manner had spectral properties similar to the enzyme isolated from milk, as shown in Table 1.

## DISCUSSION

Peroxidase activity has been found widely distributed in mammalian tissue (Bancroft and Elliot, 1934; DeGroot and Davis, 1962; Hosoya, 1962; Neufeld et al., 1958; Nickerson, Kraus, and Perry, 1957). Except in the case of myeloperoxidase isolated from leucocytes, and lactoperoxidase

TABLE 1

Comparison of Soret Maxima of Lactoperoxidase Isolated from Milk and Salivary Gland

| | Wave Length of Maximum (mμ) | |
|---|---|---|
| Form of Enzyme | (Milk) | (Steer Salivary Gland) |
| Ferric | 412 | 412 |
| Ferric cyanide | 430 | 429 |
| Ferro | 438 | 438 |
| Ferro carbon monoxide | 424 | 424 |

isolated from milk, the peroxidase activity of tissues cannot be directly attributed to a specific well-characterized hemoprotein.

In these two cases, a comparison of spectral properties has definitely established that these two hemoproteins are different. Of the other hemoprotein peroxidases, both thyroid peroxidase and tryptophane pyrrolase can be distinguished from the other enzymes by their prosthetic groups, which can be dissociated from the protein moiety (Alexander and Corcoran, 1962; Tanaka and Knox, 1959; Feigelson and Greengard, 1961).

Among the tissue peroxidases which have been described, it has been suggested by Martin et al., (1958), that the so-called "uterine peroxidase" is identical to lactoperoxidase. Rytomaa and Teir, (1961), however, have indicated that the peroxidase activity of the uterus was due to the high concentration of eosinophils in this tissue rather than to peroxidase present in uterine cells. In the present study, both the gravid and non-gravid uterus were investigated, and no evidence was obtained for the presence of lactoperoxidase in either case.

The question of the source of the peroxidase has also been raised with respect to the peroxidase in saliva. Nickerson et al., (1957), have concluded that the peroxidase activity of saliva is due to glandular tissues and not to bacteria or white cell contamination of the saliva.

The results in Figure 1 clearly show that extracts of the salivary gland of bovine tissue contain lactoperoxidase. The extracts of this tissue give agar diffusion patterns in which the band completely fuses with the band of purified lactoperoxidase. It is important to note that this enzyme appears to be present in both cow and steer salivary glands. Therefore, the presence of the enzyme is not uniquely associated with lactating mammary tissue. Lactoperoxidase could not be detected in any bovine tissue other than the salivary or mammary glands or their secretions.

As shown in Figure 1, a pig salivary gland extract also gave a band with antisera to bovine lactoperoxidase. The band, however, shows spurring, suggesting that although the salivary gland of the pig contains lactoperoxidase which is antigenically related to bovine enzyme, the two are not identical.

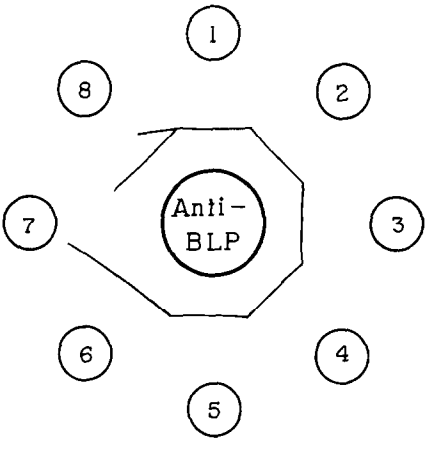


Fig. 1. Schematic diagram of agar diffusion analysis of tissue extracts. Rabbit antiserum (R76) to purified bovine lactoperoxidase contained in central well. Peripheral wells contained: 1) purified bovine lactoperoxidase; 2) crude extract of cow submaxillary gland; 3) purified lactoperoxidase from cow submaxillary gland; 4) crude extract of steer submaxillary gland; 5) purified lactoperoxidase from steer submaxillary gland; 6) extract of steer sublingual gland; 7) extract of cow uterus containing 43 mg/ml of protein; 8) extract of pig submaxillary gland.

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REFERENCES

1. Alexander, N. M., and Corcoran, B. J., J. Biol. Chem., 237, 243 (1962).
2. Allen, P. Z., and Morrison, M., Arch. Biochem. Biophys., 102, 106 (1963).
3. Bancroft, G., and Elliott, K. A. C., Biochem. J., 28, 1911 (1934).
4. DeGroot, L. J., and Davis, A. M., Endocrinology, 20, 505 (1962).
5. Feigelson, P., and Greengard, O., J. Biol. Chem., 236, 153 (1961).
6. Hosoya, T., Kondo, Y., and Ui, N., J. Biochem., 52, 180 (1962).
7. Martin, A. P., Neufeld, H. A., Lucas, F. V., and Stotz, E., J. Biol. Chem., 233, 206 (1958).
8. Morrison, M., and Hultquist, D., J. Biol. Chem., 238, 2847 (1963).
9. Neufeld, H. A., Levay, A. N., Lucas, F. V., Martin, A. P., and Stotz, E., J. Biol. Chem., 233, 209 (1958).
10. Nickerson, J. F., Kraus, F. W., and Perry, W. I., Proc. Soc. Exp. Biol. Med. 95, 405 (1957).
11. Rytomaa, T., and Teir, H., Nature, 192, 272 (1961).
12. Tanaka, T., and Knox, W. E., J. Biol. Chem., 234, 1162 (1959).