

METHOD FOR THE PURIFICATION OF LPF-HA

The present invention relates to a method for the purification of LPF-HA. More particularly, it relates to a method for producing LPF-HA (Leucocytosis-promoting Factor Hemagglutinin) from culture media of *Bordetella pertussis* in a high yield and high purity by an affinity chromatography using a denatured ceruloplasmin as a ligand.

TECHNICAL FIELD

LPF-HA is an active substance produced by *B. pertussis* phase I and phase II strains which is not produced by *B. pertussis* phase III strain having no virulence or *Bordetella parapertussis*, *Bordetella bronchiseptica*. The LPF-HA is also called as *B. pertussis* toxin and is a protein having various physiological activities. The main physiological activities are a leucocytosis-promoting activity, an insulin secretion-enhancing activity, a histamine-sensitizing activity, a hemagglutinating activity, and the like. Particularly, because of the insulin secretion-enhancing activity, it is noticed that the LPF-HA may be useful for the treatment of diabetes.

Separately from the above physiological activities, it has recently been noticed that LPF-HA shows an important function in the prophylaxis of infection of *B. pertussis* and infectious disease thereof and hence is useful as an antigen for prophylaxis of infection of *B. pertussis* [cf. Pittman, M.; Review of Infectious Diseases, 1, 401-409 (1979), and Sato, Y. et al.; Seminars in Infectious Diseases IV, Bacterial Vaccine, 380-385 (1982)].

Thus, it has been desired to develop an improved method for the separation and purification of LPF-HA simply and in a large quantity, for the purpose of studying the physiological activities of LPF-HA, of producing a medicine and of producing a pertussis vaccine having less side effect in an industrial scale.

PRIOR ART

According to known methods, the separation and purification of LPF-HA is carried out by salting out a culture medium of *B. pertussis* with ammonium sulfate, extracting and dialyzing, and then subjecting the thus obtained material to ion exchange chromatography, gel filtration [cf. Arai, H.; Biochimica et Biophysica Acta, 444, 765 (1976)] or to sucrose concentration gradient centrifugation [cf. Sato, Y.; Infect. Immun., 6, 897-704 (1972)]. According to such known methods, however, it is very hard to obtain the desired LPF-HA which shows single band in the purification analysis by electrophoresis, and its yield is very low.

In order to obtain the desired highly pure LPF-HA in a comparatively large amount, it is also proposed that a supernatant of culture media of *B. pertussis* is passed through a column packed with hydroxyapatite to adsorb LPF-HA thereon, followed by washing, eluting and then subjecting to affinity chromatography with concanavalin A-Sepharose (Con A-Sepharose, manufactured by Pharmacia) [cf. Yajima, M. et al.; J. Biochem., 83, 295-303 (1978)]. However, the affinity chromatography using concanavalin A as a ligand not only has an affinity with LPF-HA but also can adsorb saccharides, glycolipids and also other glycoproteins, and hence, it adsorbs other pertussis cell components such as F-HA (Filamentous-Hemagglutinin) and cell membrane components, which results in difficulty of isolation

of the desired highly pure LPF-HA. Thus, it is not suitable as an affinity chromatography for LPF-HA.

Since it has recently been found that human haptoglobin binds specifically to LPF-HA, it has been tried to purify LPF-HA by an affinity chromatography using as a ligand the human haptoglobin instead of the above concanavalin [cf. Iron, L. et al.; Biochimica et Biophysica Acta, 580, 175-185 (1979), and Cowell, J. et al.; Seminars in Infectious Diseases IV, Bacterial Vaccine, 371-379 (1982)]. In this case of using human haptoglobin as a ligand, there newly occurs other problem that it is necessary to take a measurement against hepatitis virus. That is, since the human haptoglobin is collected from human blood, it may be contaminated with hepatitis virus and further other unknown infectious factors. This problem is also included in case of using other animal blood. Unfortunately, however, there is no method for surely checking the contamination with hepatitis virus. It is also known that the hepatitis virus can be inactivated by heating it at 60° C. for 10 to 15 hours. It has been found by the present inventors that when haptoglobin is subjected to such a heat treatment, it loses almost the affinity to LPF-HA and hence can not exhibit the desired effect when used in the affinity chromatography.

Moreover, in case of the purification using hydroxyapatite as mentioned above, it takes a long period of time in the treatment with a column packed with hydroxyapatite, and hence, it may result in lowering of activity of LPF-HA. Thus, this method is not suitable for the purification of LPF-HA in a low cost and in an industrial scale, either.

Recently, there has also been proposed a method for collecting LPF-HA which comprises fracturing mechanically *B. pertussis* cells, extracting LPF-HA from the cell components, subjecting it to ammonium sulfate fractionation, and then, subjecting the thus obtained material to affinity chromatography using as a ligand plasma sialoproteins such as haptoglobin or ceruloplasmin, or sialoproteins such as salivary mucin (cf. British Patent First Publication 2,015,531). However, the ligand specifically disclosed in this British patent publication is merely human haptoglobin, and hence, this method still includes the problem of necessity of taking measurement for the hepatitis virus as mentioned above. Besides, the above British patent publication does not specifically mention ceruloplasmin, and it is unclear therefrom that it is effective or not. As a result of the present inventors' study, when ceruloplasmin is used as it stands, it can not exhibit sufficient effect for purification of LPF-HA.

OBJECTS OF THE INVENTION

Based on the above technical situation, the present inventors have intensively studied on an improved method for isolation and purification of LPF-HA in an industrial scale. As a result, it has been found that when a denatured ceruloplasmin is used as a ligand in an affinity chromatography, the desired purification of LPF-HA can be achieved, and further that even when it is heat-treated at 60° C. for 10 to 15 hours in order to eliminate hepatitis virus, etc., it does not lower its adsorbability of LPF-HA but rather shows increased adsorbability.

The main object of the present invention is to provide an improved method for obtaining *B. pertussis* LPF-HA in a high yield and high purity in a single step, wherein the collection of LPF-HA from culture media of *B.*